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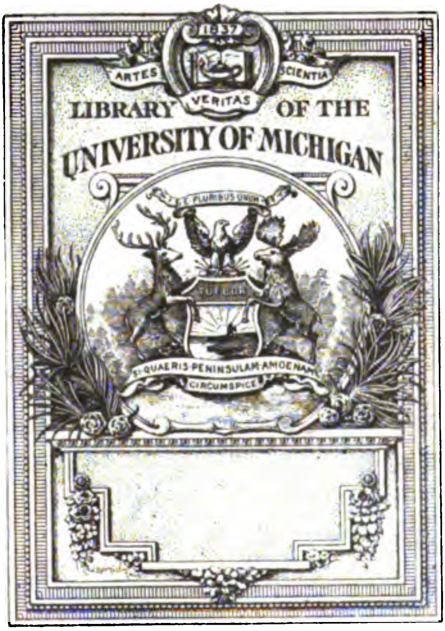
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Issued February 16, 1911.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 131.
A. D. MELVIN, CHIEF OF BUREAU.

BEEF PRODUCTION IN ALABAMA.

I. COST OF RAISING THE CATTLE.
II. WINTERING STEERS PREPARATORY TO SUMMER FATTENING ON PASTURE.
III. FATTENING CATTLE ON PASTURE.

BY

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AND

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Junior Animal Husbandman, Bureau of Animal Industry.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

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LETTER OF TRANSMITTAL.

UNITED STATES DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., October 29, 1910.

SIR: I have the honor to transmit for publication as a bulletin of this Bureau the accompanying manuscript on the subject of Beef Production in Alabama, by Prof. Dan T. Gray, of the Alabama Agricultural Experiment Station, and Mr. W. F. Ward, junior animal husbandman in this Bureau.

The work described in this paper is the result of several years' cooperative experiments between this Bureau and the Alabama Experiment Station, the main object of which was to test the possibilities of that section, and of the South generally, in regard to beef production from native stock. It can be seen from the accompanying pictures of the cattle used in these experiments that there is much room for improvement in the matter of grading up the stock for the purpose mentioned. Nevertheless the use of certain southern feeds combined with pasture proved to be highly successful with these animals, and although the work is still in progress enough has been accomplished to warrant the publication of the results to date.

The outcome of the summer feeding experiments, given in Part III of the paper, would indicate that beef animals can be very profitably prepared for market in the South. The natural advantages for cattle raising which the Southern States undoubtedly possess, especially in the tick-free portions, seem to present a powerful argument both for the eradication of the tick and for the rapid extension of the beef-cattle industry by our southern farmers. These considerations acquire additional force at the present time because of the long-continued scarcity and high prices of beef, not only in this country but abroad.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

265042

INTRODUCTORY NOTE.

The results presented in this bulletin are those of experiments continuing the investigations in beef production begun in cooperation with the Alabama Experiment Station in 1904 under the provisions of the special appropriation by Congress for investigations in animal feeding and breeding, the results of which were published as Bulletin 103 of this Bureau. The first investigation was a study of the economy of applying northern feeding systems to southern conditions. Southern feeds were used, but the cattle were kept in yards and the value of the manure was not considered. The work was carried on during three successive winters, and the results did not show sufficient profit from the methods used to warrant their continuation or extension.

The plans of the work were then changed along the lines presented in this publication, and although it is still being carried on and will be continued, sufficiently definite data have been obtained to justify publication.

Professor Gray and Mr. Ward present four good and valid reasons for raising beef cattle in Alabama, which are especially pertinent to Alabama farmers. There is a fifth reason for the extension of the beef-cattle industry, not only in Alabama but in the other Southern States, which directly concerns the country at large, namely, the absolute necessity for the country to increase its supply of beef. No more striking fact is shown by the statistics of the industry during recent years than the decrease in the supply of beef in the United States. Beef cattle appear to be decreasing in actual numbers, and much of the land formerly devoted to beef raising and fattening is being used for other purposes. As a consequence beef is high and is going higher, the amount of beef exported is falling off, and, unless some relief appears, beef will eventually become a luxury.

There are two ways of increasing the beef supply—by raising more beef in the United States or by importing it. The idea of relieving the situation by importing beef is repugnant to many, and an extension of the home industry must be found if possible. The results presented in this bulletin show that the South offers the solution. For instance, with only 40 per cent of the land of Alabama occupied, the authors show that the land is there to be used. With the tick-extermination work progressing so satisfactorily, the only serious obstacle to the industry in the South is being removed.[a]

To the close of the fiscal year 1910 an area of 129,611 square miles had been freed from ticks, and the Department is confident that complete eradication of this pest is only a matter of time. Each county placed above the quarantine line changes a territory where beef production has been a restricted industry to one of the fullest freedom of operation. To work out the economy of beef raising in this territory is one of the most pressing animal husbandry problems of the day.

Geo. M. Rommel,
Chief of Animal Husbandry Division.

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[a] It should be understood that all the cattle used in these cooperative experiments were raised and fed in quarantined territory. All were on tick-infested pastures, but excessive infestation by the cattle was prevented by greasing and dipping. The cattle therefore did not suffer the usual drain from the presence of enormous numbers of ticks. However, like all immune cattle, the protozoan which causes Texas fever was present in the blood of these cattle in probably undiminished numbers. These organisms naturally cause a serious but immeasurable drain, and there is probably no means to free cattle from them. The only known way to rid a herd of the protozoa in the blood of the cattle is to raise calves from tick-free cows and bulls on tick-free pastures. Such calves will be free from both tick and protozoa, and will, of course, be susceptible to Texas fever.

CONTENTS.

I. COST OF RAISING THE CATTLE.

Page.
Introduction 9
Details of the experiments 11
  Object of the work 11
  Cattle used 12
  Management of the herd 12
  Method of collecting data 13
  Prices of feeds 13
  Weights and gains 14
  Cost of gains 15
  Cost of wintering the cattle 16
  Area of pasture required for each animal 17
  Breeding record 18
Financial statements 19
  Feed expense to get a beef animal to various ages 19
    Extra charges and credits 20

II. WINTERING STEERS PREPARATORY TO SUMMER FATTENING ON PASTURE.

Details of the experiment 25
  Plan of the work 25
  Cattle used 26
  The winter range 26
  Method of feeding and handling the cattle 26
  Character and price of feeds used 27
Wintering the cattle 28
  Results of the winter feeding 29
  Amount of winter feeds used 30
  Gains and losses of steers by months 32
  Financial statement for winter work 33
Summary of winter work 35

III. FATTENING CATTLE ON PASTURE.

Introduction 37
The summer pasture 37
Details of the summer feeding 38
  Feeds consumed 38
  Gains made 40
  Cost of gains 41
Financial results of summer feeding 42
Slaughter results 45
Summary of summer work 46

ILLUSTRATIONS.

Page.
PLATE I. Steers at beginning of summer feeding period, 1908, lot 3, wintered on range and half ration of cowpea hay........................... 12
II. Steers at beginning of summer feeding period, 1909, portion of lot 1, wintered on range alone.. 12

BEEF PRODUCTION IN ALABAMA.

I. COST OF RAISING THE CATTLE.

INTRODUCTION.

The work of eradicating the Texas-fever tick is progressing satisfactorily in the South. Every year new areas are freed from the tick, and with the progress of the work there comes an added interest in all kinds of cattle production. When the ticks in a county are exterminated, interest begins to be immediately manifested in the beef-cattle business, as the southern farmers now realize that the Texas-fever tick has been practically the only drawback to the cattle business in the past. When the tick is finally exterminated, no section of the United States will be so well suited to beef production as the South, because of its mild climate, long grazing season, and cheap land.

At the present time the South produces but a small proportion of the meat that her people consume. In Alabama there are but 528,000 head of cattle other than milk animals,[a] or, in other words, there are about 1.2 head of cattle in the State to each family. If no outside meats were shipped into the State, this supply would be quite inadequate for the needs of the people. There is, therefore, a wide field open to the southern farmer who wishes to produce beef.

There are many reasons why the Southern States should raise more beef cattle than are being raised at the present time. First, the South, under the present system of farming, has thousands of acres of land, and good land, too, which are not being used at all. Statistics tell us that only about 40 per cent of the tillable or arable land of the South is being used. Sixty per cent of the land is lying idle and returns to the owner not a cent in wealth. All of the lands can not be used as cotton lands, because, first, there are not enough people to work the lands in any such way, and, second, many of these pauper acres are not suitable for cultivation. In fact, many acres that are now under cotton cultivation should be turned into permanent pastures and grazed with live stock. No State can become wealthy when only 40 per cent of the land capital is being used. The grocer, or the banker, or the hardware merchant could not possibly make a profit on his business if he used only 40 per cent of his capital, and the farmer can not hope to be successful in his operations until he begins to make use of at least a reasonable proportion of his capital.

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[a] Crop Reporter, February, 1910, United States Department of Agriculture.

Then, again, beef cattle should be more generally introduced, because of the good they do in building up and maintaining soils. Under the present system of cotton farming the soils are becoming poorer and poorer. With the introduction of cattle the soil will begin to improve. Director Thorne, of the Ohio Station, has been making tests with barnyard manure for several years, applying the manure upon a plat of ground upon which was running a three years' rotation of corn, wheat, and clover. Eight tons of manure an acre were applied. The average yearly increase on each acre, following the one application, was as follows:

Corn, 14.7 bushels, at 70 cents a bushel	$10. 29
Corn stover, 744 pounds, at $6 a ton	2. 23
Wheat, 8.36 bushels, at $1 a bushel	8. 36
Wheat straw, 897 pounds, at $4 a ton	1. 79
Clover hay, 686 pounds, at $12 a ton	4. 12
Total value, 8 tons of manure	26. 79
Total value, 1 ton of manure	3. 35

He further states[a] that the value of farm manure can be materially increased by balancing the manure with the addition of a carrier of phosphorus. The farm manures are too high in nitrogen as compared with the other elements. By balancing the stable manure the value of 8 tons was increased $12.20 after deducting the cost of the material used for the balancing of the manure. This is $1.53 a ton, and, when added to the $3.35 above, brings the total possible value of each ton of manure up to $4.88. During a feeding period of one hundred days each steer will produce at least 1½ tons of manure, and this profit should be added to the direct profits of feeding.

The Arkansas Station (Bulletin 68) made a test to determine the value, to each succeeding crop, of growing peas in the corn, gathering the corn, and then grazing both the peas and the stalks by the steers. The steers were being fed some cotton seed in addition to the grazing. As the result of this crop of peas and the grazing the succeeding cotton crop was increased 626.5 pounds of seed cotton per acre over the area where corn alone had been grown. A third lot was planted to corn, and the increase in corn, due to the pea crop and the grazing, was 14 bushels an acre.

A third reason why beef should be more generally produced in the South is that there is a demand for it, and the demand should be met in order that the money may be kept at home. It has been stated in a previous publication[a] that during the year 1907 there were about 15,151 home-raised animals slaughtered in the city of Birmingham (this included cattle, veal, hogs, sheep, and kids), while there were 36,097 live western animals brought into the city and slaughtered. In addition to all this, thousands of pounds of cured meats were also retailed over the city. This money should all be kept at home and added to the southern wealth. Packing houses are now being built throughout the South, and good markets are assured for the beef animals which the farmer produces.

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[a] Bulletin 183, Ohio Experiment Station.

The fourth reason offered in favor of beef production is the opportunity that it offers for the profitable utilization of "roughage." As the farmers learn the value of diversification in farming operations, there will be an increased amount of roughage, such as corn fodder, cowpea and clover hays, and soy beans, which many times can be marketed more profitably through the beef animals than in any other way. The beef cattle serve as important machines for converting the surplus fodders into valuable barnyard manure, which gives to the growing crops not only the benefits of its fertilizing elements, but enhances the natural condition of the soil by the addition of that important compound—humus. No animal can take the place of the beef steer in making use of the winter corn and cotton fields.

Finally, it may be said that beef cattle are peculiarly suited to fit into the farming operations of the South. The farms are large, and many acres are not being used because of the lack of sufficient labor. At present there is no better way to put the whole farm to work than by introducing beef cattle into the system of farming. They require but a small amount of labor in addition to that used upon the average cotton farm. The hog, while he deserves a prominent place upon almost every farm, can not be made to use all of the large uncultivated areas on the farms, as he is not strictly a grazing animal. Many farmers who have large uncultivated areas are not now sufficiently skilled in the handling of live stock to introduce sheep or dairy cattle, as these branches require more skill in stock management than the beef business. Then, too, the dairy business requires an increase in the amount of labor used upon the farm; and the labor item is one that many farmers are trying to reduce.

DETAILS OF THE EXPERIMENTS.

OBJECT OF THE WORK.

In order to obtain definite information regarding beef production in the South, which would make it possible to outline work for the future, this Bureau and the Alabama Experiment Station undertook a cooperative experiment with a large stock farmer in the Tennessee Valley.

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[a] Farmers' Bulletin 411, United States Department of Agriculture.

The primary object of this work was to learn what it would cost to raise a grade steer to the feed-lot period, under average southern conditions. After this was determined, it was expected that suggestions could be made and plans offered by which beef animals could, in the future, be produced more cheaply than were the ones in this test.

CATTLE USED.

The animals used in the work were a herd of grade Aberdeen-Angus cows, headed by two purebred Aberdeen-Angus bulls. The owner of the herd, with whom the work was conducted, began in 1900 the work of grading up some Holstein and scrub cows by the use of purebred Aberdeen-Angus bulls. The Holstein cows had been used for dairy purposes, and the scrub cows were bought from some of the neighboring farmers. The scrub and Holstein mothers were not included in the experimental work, their grade offspring being used. The experimental herd, at the beginning of the test, in 1906, consisted of the following animals:

Cows (that had dropped calves)	15
Two-year-old heifers (18 to 30 months)	13
Yearling heifers (12 to 18 months)	12
Heifer calves (recently born)	14

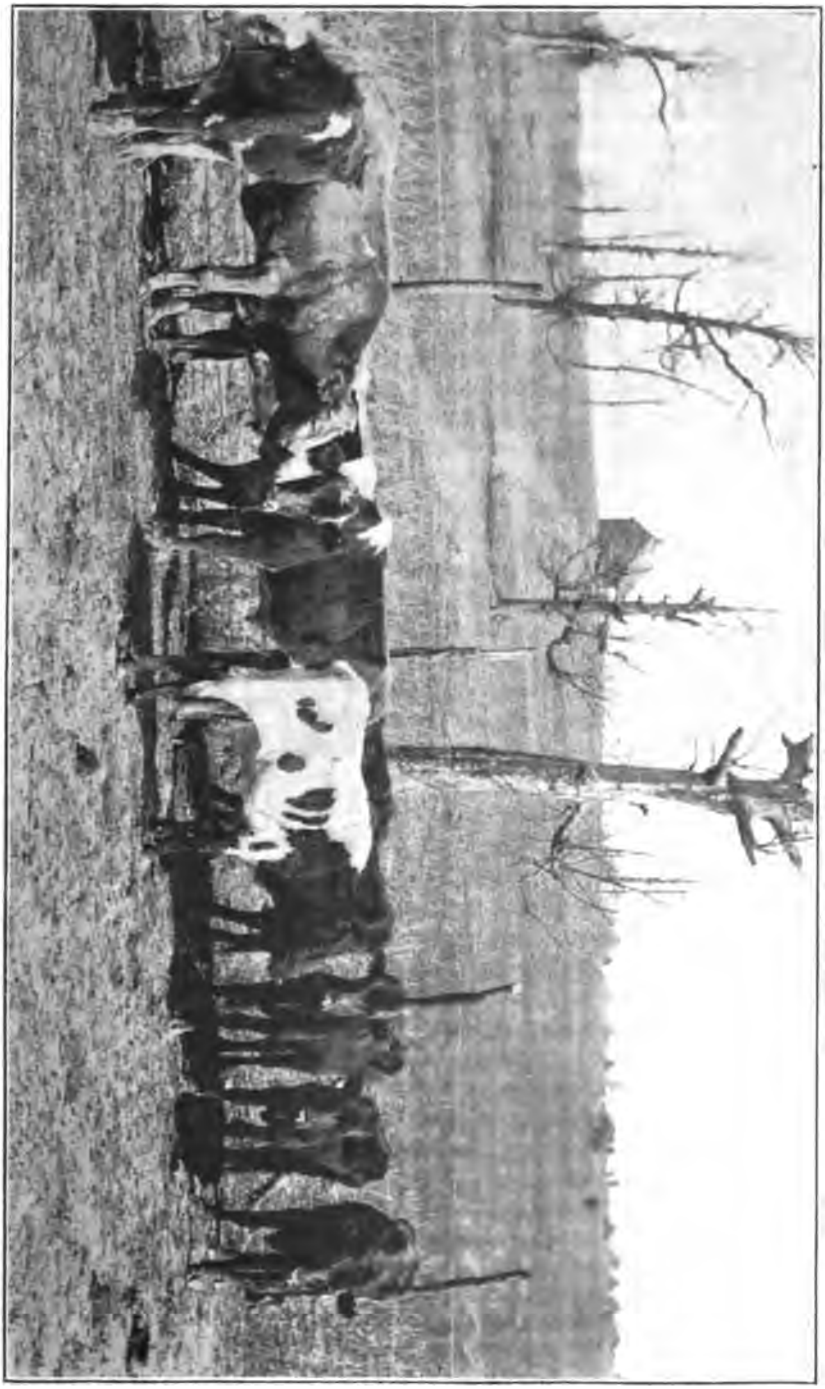
Three of the above cows were 5 years old; the others were less than 5 years of age. All of them were grade Angus. Some of the young ones were three-fourths pure, but the majority were but one-half pure. While there were some purebred Aberdeen-Angus cows upon the farm, they were not included in the test. No purebred animals were used but the bulls.

MANAGEMENT OF THE HERD.

During the summer months the herd grazed upon a good pasture; no feed was given in addition to the pasture. This pasture was made up principally of white clover, Bermuda, and Japan clover (Lespedeza). This afforded the animals abundant pasture for about seven months of the year. During the winter months all of the cattle, young and old, had the run of the range. This range, which was inclosed, consisted of the old corn and cotton fields, with some cane along the river and creek banks. In addition to the winter range, hay and cotton seed were fed (see statement later for the winter feeds), so when spring came the cattle were in reasonably good flesh. It might have been profitable to have kept all the animals gaining throughout the winter months; as to this the authors can not say, since this test does not cover that point. The young stock did make gains during each winter, but the cows and older animals usually lost in weight during the latter part of the winter.

Steers at Beginning of Summer Feeding Period, 1908.

Lot 3, wintered on range and half ration of cowpea hay.



STEERS AT BEGINNING OF SUMMER FEEDING PERIOD, 1909.

Portion of lot 1, wintered on range alone.

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The shelter was of such a nature that none of the animals suffered from the cold. During the mild winter weather the cane-brake afforded ample protection from the cold, and in severe storms or continued wet spells the cattle barn was used.

During the first year of the test the bulls were not allowed to run with the cows. They were kept in a pasture separate from the cows, and the cows brought there to be bred. It was learned, however, that many of the cows went through the year without bringing calves, as the other farm work prevented the owner from keeping in close touch with them. During the second year's work the bulls were allowed to run with the cows; thereupon the percentage of calves born during the first quarter of the year increased very materially, as compared with the previous year.

As previously mentioned, no effort was made to eradicate the ticks completely. When the cattle became badly infested they were greased on those parts of the body where the ticks were most numerous.

METHOD OF COLLECTING DATA.

The farm was visited at least once every three months by a bureau or station representative, and data secured about the births, deaths, weights, feeds used, and other items. Each animal was numbered by means of a metal tag in the ear, so that individual records could be secured. Soon after a calf was born it was tagged and weighed. All feeds were weighed or measured out to the animals. Large amounts of manure were produced but no account was kept of it, as most of it was dropped out in the fields and pastures. During the winter months some manure was collected around the barns and lots; this was all hauled onto the cultivated fields.

PRICES OF FEEDS.

Local conditions determined to a large extent the price of feeds. Any prices that the authors might assume would not meet all conditions, but the following have been used as a basis for the financial estimates:

Mixed hay	per ton	$6.00
Cotton seed	do	14.00
Green sorghum	do	1.50
Pasture for the season	per acre	2.50

The hay, which consisted of a mixture of sorghum, crab grass, Johnson grass, and cowpeas was not of good quality, so a rather low farm price was placed upon it. Six dollars a ton was all it was worth. The green sorghum was used one fall (1906) for several days to supplement a pasture which was rendered short on account of an extreme drought and an early frost. The sorghum was cut and immediately thrown to the cattle. No price was placed upon the winter range. One hundred sheep, and about 30 horses and mules used the winter range in common with the cattle.

WEIGHTS AND GAINS.

TABLE 1.—*Average weights and gains of the cattle.*

Class.	Number of animals.	Average final weight.	Summer gains.		Winter gains.		Summer and winter gains.	
			Average gain per head for summer.	Average daily gain.	Average gain (+) or loss (−) per head for winter.	Average daily gain (+) or loss (−).	Total gain per head.	Daily gain per head.
		Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
1 to 12 months	44	402	224	1.24	+ 52	+0.29	276	0.77
12 to 24 months	42	645	219	1.22	− 16	− .09	203	.56
24 to 30 months	30	773	196	1.09	− 25	− .14	170	.47
24 to 33 months	18	832	170	.95	− 35	− .19	136	.40
Cows[a]	....	....	116	.65	−104	− .58	12	.03

[a] The number of cows is not stated because the number varied from time to time.

From the above table it is seen that at 12 months of age the calves averaged 402 pounds in weight, while the 24-months-old steers averaged 645 pounds, the 30-months-old steers averaged 773 pounds, and the animals which were 33 months old weighed 832 pounds. These are light weights, which were due, in part at least, to the fact that the animals had ticks on them during the summer months.

During the summer the gains were heaviest with the calves, as they made a gain of 224 pounds from April 15 to October 15, or a daily gain of 1.24 pounds. The yearlings made a gain of 219 pounds, or a daily gain of 1.22 pounds during the summer six months. During the third period (24 to 30 months) a daily gain of 1.08 pounds was made, while in the fourth period (24 to 33 months) a daily gain of only 0.95 of a pound per head was made. The cows gained 0.65 of a pound per head per day, or a total gain of 116 pounds each during the summer.

During the winter months (October 16 to April 14) each calf made a total gain of 52 pounds, or a daily gain of 0.29 of a pound per head. The yearlings lost 16 pounds each in weight for the winter, the animals ranging from 24 to 30 months old lost 25 pounds each; and those which came in the fourth class (24 to 33 months) lost 35 pounds each for the same period. The cows lost 104 pounds each, which was largely due to the fact that many of them dropped calves during the winter time.

An average of the whole year shows that the calves made a daily gain of 0.77 of a pound, or a total gain of 276 pounds per head. The yearlings made a daily gain of 0.564 of a pound per head, or a total of 203 pounds for the 12 months. A gain of 170 pounds per head, or a daily gain of 0.47 of a pound, was made by the cattle while they were increasing from 24 to 30 months in age, while 136 pounds, or 0.4 of a pound per head per day, was made by the animals while they were increasing in age from 24 to 33 months. The cows gained only 12 pounds for the year, showing that they were practically mature when they first dropped calves.

In short, the table shows that under the conditions of this experiment the daily gains were smaller as the animals increased in age. This was true in both the summer and the winter work.

The gains were not as large as they should have been, due partly to the extremely dry weather from June to July 20, 1906, when the grass in the pastures became perfectly dry, and partly to the fact that one of the pastures used in 1907 was so wet in the early spring that the grass did not grow satisfactorily during the whole summer. The fact has already been mentioned that the animals were also infested with the cattle tick.

COST OF GAINS.

The cost of gains during the summer was based upon a rental of $2.50 per acre for all land used for pasture; calves under 1 year of age were charged one-half price. When the cost of keeping the dam was not charged against the calves, they made 100 pounds of gain at a cost of 63 cents, but when the expense of pasturing both dam and calf was charged against the calf, the cost of 100 pounds of gain was raised to $1.88. The cost of keeping the dam represents the expense of keeping 1.39 cows, as only 1 calf was produced to every 1.39 cows (see Table 4). The yearlings made 100 pounds of gain during the summer at a cost of $1.28, the 2½-year-old steers (24 to 30 months) at a cost of $1.43, and the gains of the steers in the fourth class (24 to 33 months) were made at a cost of $1.65 per 100 pounds.

In the winter all cattle had access to the corn, cotton, and pea-stubble fields of the plantation and were fed some hay and a small amount of cotton seed to keep them in a fair condition of flesh. The average cost of wintering those animals that were more than 12 months of age was $4.90 per head (see Table 2).

When the cost of wintering the dam was charged against the calf, the cost of 100 pounds of gain was $9.42. As all the other animals lost some in weight during this period, the cost of gain could not be determined.

The last column of Table 2 shows that when the cost of keeping the dam was not charged against the calf, 100 pounds of gain for the whole year cost 51 cents, but when the dam's yearly expense, as well as the pasture of the calf, was charged against the calf, the total cost to make 100 pounds of gain was $3.30. The cost of keeping an animal from the time he was 12 months until he was 24 months old was $7.70; the amount of gain was 203 pounds, thus making 100 pounds of gain cost $3.79.

The 2-year-old animals (24 to 30 months) gained 170 pounds at a a cost of $7.70, or at a rate of $4.53 per 100 pounds for the year. The cost of gain on the long 2-year-old cattle (24 to 33 months) was $5.66 per 100 pounds. The last column of the table points out the fact that, as the animal advanced from the calf period to maturity, the cost of 100 pounds of gain increased, and, all other conditions being equal, the younger the animal the cheaper were the gains.

The results shown in Table 2 are based upon the charges for feed as shown on page 13.

TABLE 2.—*Average cost of summer, winter, and annual gains.*

Class.	Summer.			Winter.			Whole year.		
	Pounds gain.	Cost of pasture.	Cost of 100 pounds gain.	Pounds gain (+) or loss (−).	Cost of feed.	Cost of 100 pounds gain.	Pounds gain.	Cost of pasture and feed.	Cost of 100 pounds gain.
Calves,[a] 1 to 12 months...	224	$1.40	$0.625	+ 52	....	....	276	$1.40	$0.51
Calves,[b] 1 to 12 months...	224	4.20	1.88	+ 52	$4.90	$9.42	276	9.10	3.30
Yearlings, 12 to 24 months.	219	2.80	1.28	− 16	4.90	....	203	7.70	3.79
2-year-olds, 24 to 30 months..................	195	2.80	1.43	− 25	4.90	....	170	7.70	4.53
2-year-olds, 24 to 33 months..................	170	2.80	1.65	− 35	4.90	....	136	7.70	5.66
Cows, all ages............	116	2.80	2.41	−104	4.90	....	12	7.70	64.18

[a] Keep of dam not charged. [b] Keep of dam charged.

COST OF WINTERING THE CATTLE.

Table 3 shows the total amounts of feed consumed, the total cost to winter the whole herd, and the average cost per winter for three consecutive winters. Only animals above 1 year old are counted. The feed that the calves ate is charged against those animals which were more than 1 year old.

TABLE 3.—*Cost of wintering cattle.*

Year.	Number of cattle.	Pounds of cotton seed consumed.	Cost of cotton seed.	Pounds of green sorghum consumed.	Cost of sorghum.	Pounds of hay consumed.	Cost of hay.	Total cost.	Average cost per head.
1905–6..............	45	27,000	$189.00	....	....	35,600	$106.80	$295.80	$6.57
1906–7..............	65	12,000	84.00	22,000	$16.50	39,600	118.80	219.30	3.37
1907–8..............	59	3,132	21.92	....	....	86,443	259.32	281.24	4.77
Average..............	....	14,044	98.31	7,333	5.50	53,881	161.64	265.45	4.90

During the winter of 1905–6 the herd consumed 27,000 pounds of cotton seed and 35,600 pounds of hay. That is, each animal that was more than 12 months old consumed 600 pounds of cotton seed and 791 pounds of hay for the whole winter. The winter of 1905–6 was a hard one on the cattle, as it was very wet and rainy, and the winter range was not as good as usual, hence the large amount of feed consumed.

With cotton seed charged at $14 a ton and hay at $6 a ton, each animal that was over 12 months old consumed $6.57 worth of feed. The cattle, as a herd, came through the winter in good condition.

The winter of 1906–7 was very mild and the cattle did not eat as much feed per head as they did the previous winter. There were two very heavy frosts on the nights of October 13 and 14, however, which killed all the Japan clover (Lespedeza), so the cattle had to be fed some green sorghum from the middle of October until the fields became available as winter range. Each animal consumed, during the whole winter, 338 pounds of green sorghum, 185 pounds of cotton seed, and 610 pounds of hay. The cost of wintering each animal over 12 months old was $3.37.

From October 16 to December 1 of the winter 1907–8 the cattle were fed hay and a small amount of cotton seed, as there was no green sorghum to be used. During this time they consumed 11 pounds of hay and 1.2 pounds of cotton seed per head per day. From December 1 to January 1 they were in the fields and canebrake and did not come up for feed. From January 1 to March 20, 1908, the cattle came up to the barn each evening and were fed hay, but no grain. For the whole winter each animal consumed 53 pounds of cotton seed and 1,465 pounds of hay. The cost of wintering was $4.77 per head.

The average for the three winters shows that it cost $4.90 to winter each animal over 12 months of age.

AREA OF PASTURE REQUIRED FOR EACH ANIMAL.

A number of cattle not in the experiment were grazed in the pasture with the experimental cattle. Assuming that two calves would eat as much grass as an animal over 12 months old (and this assumption is followed out in rental charges), there would be the equivalent of 92 animals on 103 acres of land during the summer of 1906, or an average of 1.12 acres of pasture to each animal. During the year 1907 there were 90 animals on the 103 acres, giving an average of 1.14 acres to each animal. An average for the two years shows that 1.13 acres of land furnished pasture for one animal. This area, when charged at $2.50 per acre, gives a cost of $2.80 per season for the pasture of each animal over 12 months old.

This pasture was far better than the ordinary Alabama pasture, as is shown by the fact that 1.13 acres supplied sufficient pasture for one animal. On the average, from 3½ to 5½ acres are required for each animal. When this piece of land was first put down to pasture it would not keep as many animals as it does now; in fact it was no better than the average pasture, but by grazing it has been raised to its present state of fertility.

BREEDING RECORD.

TABLE 4.—*The percentage of calves born.*

Year.	Number of cows.	Calves dropped.		Record by quarters; number of calves dropped.			
				January 15 to April 14.	April 15 to July 14.	July 15 to October 14.	October 15 to January 14.
		Number.	*Per cent.*				
1906	24	17	70.8	5	4	5	3
1907	25	18	72	9	1	5	3
1908	25	[a] 14	....	14	....	....	....

[a] The experiment closed on April 14, 1908, so no record was obtained later than this date.

An animal that had dropped a calf was classified as a cow; the heifers were put in this class as soon as they calved. The number of calves born in 1906 and 1907 was very small compared to the number of cows that should have brought calves. This low number was partly due, no doubt, to the fact that the bulls were kept away from the cows, and the owner, owing to the pressure of other business, was not able to breed the cows when they should have been bred. The owner soon realized the fact that too many of the cows went through the year without bringing calves, so in the spring of 1907 he turned the bulls with the cows and permitted them to run together the entire year.

A complete record of the number of calves dropped was not secured for the year 1908—the year after the bulls were turned with the cows—as the test closed in April; but during the first quarter 14 calves were born, while during the same quarter of the years 1906 and 1907 only 5 and 9 calves, respectively, were born. No record was kept of the number of calves dropped after April, 1908, but when the last notes and weights were made it was seen that practically all of the cows were pregnant. It is, of course, a disputed point whether it is better to allow the bull to be with the cows or to keep him away from them all of the time. The farmer who has large pastures and much other business to look after, in addition to the cattle, can not possibly obtain a high percentage of calves unless the bull is permitted to run with the cows. The busy farmer will not see the cows at the right time. However, the breeder of registered animals should not allow the bull to run with the cows, as it is desirable that in such cases a record of the date of service be kept.

It is important that as many of the cows as possible produce calves each year; the idle cow is not only idle capital, but she is a constant consumer of farm products. The idle cow has a very important part to play in the total expense of raising a calf, as the expense of keeping her must be charged against the calves which other cows produce (see financial statement below). When there were 25 cows the owner had $750 invested in cattle (estimating each cow to be worth $30); of this amount only $540 was returning a profit when 72 per cent of the cows brought calves. In this case there were $210 invested in idle cattle; this amount represents the equivalent of 7 cows, and those 7 cows consumed $51.80 worth of feed in a year.

FINANCIAL STATEMENTS.

FEED EXPENSE TO GET A BEEF ANIMAL TO VARIOUS AGES.

As a rule the farmer charges nothing against the cost of raising a calf but the feeds consumed. Looking at it from this standpoint the cost of raising a calf, in this experiment, to various ages was as follows:

TABLE 5.—*Cost of raising a calf to various ages, charging feed only.*

(A) To 12 months:	
To winter feed of 1.39 cows the first winter	$6.81
To summer pasture of 1.39 cows	3.89
To summer pasture of 1 calf	1.40
Total cost	12.10
Cost per hundredweight	3.01
(B) To 24 months:	
To cost of 12-months-old calf	12.10
To winter feed of animal (12 to 24 months)[a]	4.90
To summer pasture (12 to 24 months)	2.80
Total cost	19.80
Cost per hundredweight	3.07
(C) To 30 months:	
To cost of 24-months-old steer	19.80
To winter feed of animal (24 to 30 months)[a]	4.90
Total cost	24.70
Cost per hundredweight	3.20
(D) To 33 months:	
To cost of 30-months-old steer	24.70
To summer pasture for one-half summer	1.40
Total cost	26.10
Cost per hundredweight	3.14

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[a] It is assumed that all animals over 12 months of age ate the same amount of feed, which assumption is, of course, not absolutely accurate. In rental practice, however, this assumption is carried out.

It should be understood that the different classes of animals were not kept separate and fed in different lots. They all ran together, so the above statement is only a close approximation of the cost of raising the animals to the various ages. It was assumed that all animals over 12 months of age ate the same amount of feed and pasture; it was further assumed that the animal under 12 months of age used only one-half as much pasture as the animal that was over 1 year old. The last assumption is in keeping with actual farm charges for pasture. It should be stated, also, that the cost to winter an animal was arrived at by dividing the total number of animals over 1 year old into the total cost of the feed consumed during the winter months.

When placing the value upon the feeds, as shown on page 13, it cost $12.10 to raise a 12-months-old calf, $19.80 if he was kept until he was 24 months of age, $24.70 to raise him to 2½ years old, and $26.10 to keep him until he was 33 months old. In other words, it cost about 3 cents a pound to grow the animal to various ages, when nothing but the feed and pasture was charged against him.

This feed bill could be materially reduced by extending the pasture grazing season. The pasture season could be extended at least two months, almost anywhere in the South, by the use of "spotted" bur clover (*Medicago maculata*). Bur clover is a winter-growing crop and occupies the ground in common with Bermuda grass, which makes its growth during the summer months.

EXTRA CHARGES AND CREDITS.

In estimating the cost of producing a beef animal it is usual to charge nothing against the animal but the winter feed and the pasture used. But there are other items that should be included, such as interest on the money invested in the cattle, mortality, depreciation in value of the cows, and other items. On the other hand, he should be credited with the manure produced. The following estimates charge the steer not only with the feeds used but the other items just mentioned, and give him credit for the approximate amount of manure produced:

TABLE 6.—*Cost of raising a calf to various ages. Total charges and credits.*

(A) To 12 months old:	
To winter feed of 1.39 cows	$6.81
To summer pasture of 1.39 cows	3.89
To summer pasture of calf	1.40
To 7 per cent interest on 1.39 cows at $30 per head	2.92
To 7 per cent interest on $\frac{1}{25}$ of a bull worth $150	.42
To annual depreciation in value of 1.39 cows, at $1.50 each	2.09
To pro rata depreciation of herd bull	.80

(A) To 12 months old—Continued.

To taxes, insurance, fencing, and repairs		$0.86
To 4 per cent mortality of 1.39 cows at $30 each		1.67
To 4 per cent mortality (insurance) on $\frac{1}{25}$ of bull		.24
Total		21.10
By 3,600 pounds of calf manure[a] at $1.25 a ton	$2.25	
By 15,012 pounds of cow manure (1.39 cows) at $1.25 a ton	9.38	
		11.63
Total expense of calf		9.47
Cost per hundredweight		2.35

(B) To 24 months old:

To cost at 12 months of age (manure not included)	21.10
To winter feed	4.90
To summer pasture	2.80
To 7 per cent interest on yearling	1.48
To taxes, insurance, repairs, etc	.86
To 4 per cent mortality	.84
Total	31.98
By 27,612 pounds of manure for 24 months at $1.25 a ton	17.26
Total expense of steer	14.72
Cost per hundredweight	2.28

(C) To 30 months old:

To cost at 24 months of age (manure not included)	31.98
To winter feed	4.90
To 7 per cent interest on 2-year-old animal for 6 months	1.12
To taxes, insurance, repairs, etc., for 6 months	.43
To 4 per cent mortality of 2-year-old animal for 6 months	.64
Total	39.07
By 33,012 pounds of manure for 30 months at $1.25 a ton	20.63
Total expense of steer	18.44
Cost per hundredweight	2.39

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[a] The totals in this table are computed on the basis of a year containing twelve 30-day periods, or 360 days in all. In estimating the amount of manure produced it was assumed that the animal under 1 year of age produced 20 pounds a day for 180 days; that the yearling produced 25 pounds a day for a year; and that the 2-year-old steer and the cow each produced 30 pounds a day. The price of manure, $1.25 a ton is an assumed one, as there was no way to determine its exact value. But, judging from the many tests that have been made at experiment stations, the above value is a very conservative one. For instance, as quoted in the introduction to this paper, the Ohio experiments showed raw manure to be worth $3.35 a ton when placed under the crops mentioned, and when the manure was treated with a phosphorous carrier its value was raised to $4.88 a ton. There was a difference, though, between the Ohio manure and the manure secured in the above tests; the Ohio manure was collected in the winter time when grains and hays were fed. It was a richer manure than that made during the summer months in this test, but probably no richer than the Alabama manures made during the winter months.

(D) To 33 months old:

To cost at 24 months old (manure not included)	$31.98
To winter feed	4.90
To 3 months pasture	1.40
To 7 per cent interest on 2-year-old animal for 9 months	1.68
To taxes, insurance, repairs, etc., for 9 months	.64
To 4 per cent mortality for 9 months	.96
Total	41.56
By 35,712 pounds of manure for 33 months at $1.25 per ton	22.32
Total cost of steer	19.24
Cost per hundredweight	2.31

It is seen that when a calf is charged with everything that could be charged against him, and then credited with the manure produced, the cost of making 100 pounds of gain was somewhat smaller than the figures obtained when nothing but the feed and pasture were taken into consideration.

The labor employed to feed and look after the animals was not included in the above estimate, as it was a very small item. One winter the labor to feed and care for the cattle was $10 for the whole herd. Another winter the total labor item was only $7.50. The method used in the feeding and handling involved the use of but little labor; the feeding was done but once a day, when the cotton seed and the hay were measured out to the cattle in a very few minutes.

When all of the expenses were charged against the animals and no credit was made for the manure, the expense of producing a steer varied from $4.96 to $5.25 per hundred pounds, as follows:

To 12 months of age, $5.25 per hundredweight.
To 24 months of age, $4.96 per hundredweight.
To 30 months of age, $5.05 per hundredweight.
To 33 months of age, $5.00 per hundredweight.

These figures mean that if the animals are sold for the above prices[a] the feeds used are marketed at a good farm price; all deaths are deducted; 7 per cent interest is received on the money invested in the animals; $2.50 an acre is secured as rent for the summer pasture; and finally the manure is secured free. Of course, in order that all these profits may be realized good cattle must be raised, it can not be done with scrubs; the scrub will not sell to advantage when he is offered to the butcher or packer, as his meat is of poor quality and he dresses out a low per cent of salable meat.

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[a] These prices can be realized for good cattle as is shown by the fact that 60 steers, of about the same quality as those in this test, were fed by the Bureau of Animal Industry and the Alabama Experiment Station and sold February 28, 1910, on the Louisville market for $5.75 per hundredweight.

It may be remarked, also, that the cattle upon this farm were not produced as cheaply as it is possible to raise them in the South. At least two farm practices can be introduced upon the average farm that will make it possible for steers to be raised much cheaper than was the case with these animals. In this test no winter pastures were used, except the winter range. Through the use of a combination of bur clover and Bermuda grass the pasture season can be extended at least two months in the year. The farmer who lives as far south as Greenville, Ala., can have a grazing pasture the year through by the use of Bermuda grass, bur clover, and velvet beans. In the second place the cattle were infested with the Texas tick, which reduced their average size considerably. Through the combined efforts of the Federal Government and the Southern States the tick is now being exterminated, and when this is accomplished the farmer can expect to raise larger cattle than formerly.

II. WINTERING STEERS PREPARATORY TO SUMMER FATTENING ON PASTURE.

Before proceeding with the feeding experiment which will be described in this and the succeeding part of this bulletin, it is desired to emphasize one point that was brought out very clearly in connection with the work reported in Part I, namely, that if money is to be made in raising beef for the market the steer must be properly finished before he is offered for sale. If the steer is sold unfinished, the man who raised him is almost sure to lose money on the operation.

The question arises, then, How shall the steer, after he has been raised, or has reached the feed-lot period, be finished for the market to get the greatest possible profit out of him? The steer can be finished in one of two ways; he can be fattened during the winter months, or he can be fattened during the summer months while the pastures are available. Since the cooperative work between the Bureau of Animal Industry and the Alabama Experiment Station began, some results have been published relative to winter fattening.[a] The results of two years' work in fattening cattle upon pasture during the summer months and selling the cattle at the end of the summer are here presented, dealing first with the general plan of the work and the wintering of the steers. It should be understood that this bulletin is only a report of the progress of the work, as the experiments are being continued.

DETAILS OF THE EXPERIMENT.

PLAN OF THE WORK.

The cattle were bought in the fall on account of the fact that they could be bought much cheaper in the fall than in the spring. In fact, they could hardly be bought at all in the spring. But they were not to be fattened until the following summer, so it became necessary to make a study of the cheapest and best methods of getting these mature steers through the winter months. The work was therefore divided into (1) a study of methods of wintering mature steers, and (2) fattening these steers on pasture the following summer. The cattle used in the winter's work were continued into the following summer's work.

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[a] Bureau of Animal Industry Bulletin 103.

Owing to the fact that pasture was not available upon the experiment station at Auburn, Ala., the work was carried on elsewhere upon farms whose owners kindly agreed to cooperate in the work. The winter range and summer pastures on these farms were divided into lots suitable for the work. One of the authors of this bulletin, Mr. W. F. Ward, was stationed upon the farm and had personal supervision of all the experimental work.

At the end of each experiment the cattle were all shipped to the New Orleans market, where complete sale and slaughter records were secured.

CATTLE USED.

The various pictures will show the kind of cattle which were used in these tests. Grade Aberdeen-Angus, Shorthorn, Hereford, and Red Polled were used, but many of them had a predominance of Jersey and scrub breeding. They were all bought of farmers in Sumter, Wilcox, Marengo, and neighboring counties, so they represented the average cattle of the western part of Alabama. They varied from 2 to 4 years of age. As will be seen later, the average weight at the beginning of the fall work was about 750 pounds each.

THE WINTER RANGE.

The winter range consisted of the winter corn and cotton fields. The leaves had not been stripped from the cornstalks. Crab grass had grown up sufficiently between the rows of corn, after the last cultivation, to be of some value to the cattle during the early weeks of the winter. No canebrakes were used. Of course the man who has canebrakes has an advantage in handling and feeding cattle in the winter time. The cattle, except those in the range lots, were not given unlimited range; each lot was confined to a certain area. Those animals which were confined in limited areas had about 10 acres each upon which to graze. The outside cattle or range lot had an unlimited grazing area. The winter range was available for use immediately after all the cotton had been picked.

METHOD OF FEEDING AND HANDLING THE CATTLE.

In both the winter and the summer work the steers were fed but once a day. In the winter time movable feed troughs were placed out in the fields in which to feed the hulls, cotton-seed meal, and cotton seed, and movable hay racks were made in which to feed the hay. The racks and troughs were all made movable, so that the manure would be distributed over the corn and cotton fields. Movable troughs were also used during the summer feeding on pasture. No feeds were thrown upon the ground.

No shelter, except trees, was provided for the cattle in either the winter or summer time. They had no access to sheds. They did not suffer to any appreciable extent from the cold in the winter time or from the heat in the summer time. The summer pastures were well provided with good shade trees. When a summer shade is provided, cattle will suffer no more from heat in Alabama than they will in Illinois or Iowa.

While there were ticks in the pastures, the cattle were not permitted to become badly infested with them; a dipping vat was used to keep down heavy infestation. In the two years' work, during which time over 300 head of cattle were fattened, there were only four cases of Texas fever, and none of these cases was lost. In future work it is expected that the tick will be entirely eliminated.

The weight of each steer was secured at the beginning and end of each test, and the total weight of each lot was noted every twenty-eight days. When the steers were sold they had to be driven 9 miles to the shipping point, Scooba, Miss.

CHARACTER AND PRICE OF FEEDS USED.

When the feeds were purchased upon the market the expense of hauling to the farm was added to the market price and used in making up the financial statement. When the feed used was grown upon the farm an assumed market price was placed upon it. Local conditions determine, to a large extent, the farm prices of feeds. Any prices that might be assumed would not meet all conditions, but the following prices have been taken as a basis upon which to make the financial estimates:

Cotton-seed meal	per ton..	$26.00
Cotton seed	do....	14.00
Cotton-seed cake	do....	25.00
Cold-pressed cotton-seed cake	do....	23.00
Cotton-seed hulls	do....	6.00
Damaged hay	do....	5.00
Cow-pea hay	do....	10.00
Pasture, per steer	per month..	.50

The above represents the prices of the purchased feeds laid down on the farm; the farm was 15 miles from the railroad station (Epes, Ala.) where the feeds were secured. The cotton-seed cake had been broken into nut size and sacked. This cake can be purchased in the large cake size, just as it comes from the press, for about $2 a ton cheaper than in the nut size. Some feeders find that it pays to break the cake on their own farms. The cake is the same thing as the cotton-seed meal, except that it is not ground into a meal. There are several advantages in feeding cake in place of cotton-seed meal, especially in summer feeding. A rain does not render the cake unpalatable, but will often put the meal in such a condition that the cattle will not eat it. Again, no loss is incurred with the cake during windy days; whereas the meal, when fed in the open pasture, is sometimes wasted on account of the winds. Furthermore, the cake requires chewing before being swallowed, and therefore must be eaten very much more slowly than the meal, so when a number of steers are being fed together the greedy one has little chance to get enough cake to produce scours. In feeding cotton-seed meal the greedy steer often scours because he can bolt the meal and get more than his share; this not only injures the steer but makes the bunch "feed out" unevenly.

The cold-pressed cotton-seed cake is a special cake which consists of everything in the seed except the oil. It is the residue after the oil has been extracted from the cotton seed by the cold process. These tests do not show it to be as valuable for feeding purposes as the ordinary cotton-seed cake. The chemical analysis of the cake fed, as reported by the State chemist, B. B. Ross, of Auburn, was as follows:

	Per cent.
Moisture	9.75
Ash	4.70
Fiber	21.18
Protein	27.62
Ether extract (fat)	8.78
Carbohydrates	27.97

For comparison with the above we insert the analysis of cotton-seed meal as given in Henry's Feeds and Feeding, as follows:

	Per cent.
Moisture	8.2
Ash	7.2
Fiber	5.6
Protein	42.3
Ether extract (fat)	13.1
Carbohydrates	23.6

The mixed hay was a second or third class hay that could not be sold upon the market at all. It consisted of a mixture of Johnson grass, crab grass, and some alfalfa. The price placed upon it was all it was worth. The cow-pea hay was bright and of good quality.

WINTERING THE CATTLE.

Farmers are not agreed as to what is the best way to handle and feed mature steers during the winter months. Some farmers claim that the animals should be "roughed" through the winter upon a very small amount of feed in addition to the winter range; some hold that the range needs no supplementary feed at all; still others believe that the steer should be fed liberally so that he will be kept gaining all through the winter months.

The cattle used in the winter work were dehorned, tagged, and divided into lots (three lots in the winter of 1907–8 and four lots in the winter of 1908–9), so that a study could be made of the amount of feed that should be fed during the winter time, and also to learn the value of some of the southern feeds for carrying cattle through the cold months.

RESULTS OF THE WINTER FEEDING.

The winters of 1907–8 and 1908–9 were both mild ones. There was no weather cold enough to make the steers suffer, although, as before mentioned, there was no shelter except a few trees. The following table shows the rations fed, total weights, and gains of each lot for the two winters:

TABLE 7.—*Results of winter feeding.*

WINTER OF 1907–8 (84 DAYS).

Lot.	Number of animals.	Ration.	Average initial weight of each steer.	Average final weight of each steer.	Average gain (+) or loss (−) of each steer.	Average daily gain (+) or loss (−) of each steer.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
1	26	Range alone	722	625	−97	−1.15
2	29	Range plus half ration cotton-seed meal and hulls	726	720	− 6	− .07
3	24	Range plus half ration cow-pea hay	724	715	− 9	− .11

WINTER OF 1908–9 (98 DAYS).

Lot.	Number of animals.	Ration.	Average initial weight of each steer.	Average final weight of each steer.	Average gain (+) or loss (−) of each steer.	Average daily gain (+) or loss (−) of each steer.
1	25	Range alone	705	599	−106	−1.08
2	25	Range plus half ration cotton-seed meal and hulls	705	708	+ 3	+ .03
3	25	Range plus half ration cotton-seed	706	666	− 40	− .40
4[a]	25	Range plus half ration cheap hay	689	649	− 40	− .57

[a] This lot started January 1, so fed only 70 days.

It was intended that the steers which received some feed in addition to the range should suffer no loss in weight during the winter months, but in some cases the loss was considerable during the latter part of the winter period when the range afforded very little grazing. The object was to give just enough feed, in addition to the range, to enable the cattle to hold their fall weight. No gains in live weight were desired. It should be remembered that these were all practically mature cattle, varying in age from 2 to 4 years.

During the first winter the experiment continued from December 10 to March 2, a period of eighty-four days. During this time the range cattle (lot 1) lost 97 pounds each in live weight, while the steers in lots 2 and 3 practically held their fall weights. All of the cattle came through the winter in excellent health, and while those in the range lot were thin, they were in good condition for grazing; they evidently had not been weakened in any way. At the opening of spring the steers in the cow-pea hay lot (lot 3) seemed to be in better thrift than those in lot 2 (the cotton-seed meal and hulls lot), although they made practically the same gains in weight. The hay used in 1907–8 was good in quality.

During the winter of 1908–9 the test continued from December 4 to March 11, a period of ninety-eight days. There were practically the same losses as the previous winter in lots 1 and 2. The steers in lots 3 and 4 lost rapidly in weight the last month of the test, due to the fact that the grazing on the range was not good at the end of the season; it was not intended that they should shrink in weight. Lot 4 was not started in the test until January 1, so the cattle in this lot were fed only seventy days. The hay used in lot 4 was a very cheap hay; it was made up of a mixture of Johnson grass, crab grass, and some alfalfa, but had been damaged by rain to such an extent that it could not be sold at all.

AMOUNT OF WINTER FEEDS USED.

During the first winter (1907–8) a comparison was made between feeding on the range alone and the same when supplemented in one lot with a part ration of cotton-seed meal and hulls, and in a third lot with a good quality of cow-pea hay. The following winter (1908–9) the same comparison was again made as regards lots 1 and 2, while in a third lot cotton seed, and in a fourth lot some damaged mixed hay were used to supplement the range.

TABLE 8.—*Quantity of winter feeds used.*

WINTER OF 1907–8 (84 DAYS).

Lot.	Number of steers in lot.	Ration.	Total amount consumed per steer.		Daily amount feed consumed per steer.	
			Concentrates.	Roughage.	Concentrates.	Roughage.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
1	26	Range alone	None.	None.	None.	None.
2	29	Range plus half ration cotton-seed meal and hulls	197	714	2.35	8.5
3	24	Range plus half ration cow-pea hay	None.	714	None.	8.5
		WINTER OF 1908–9 (98 DAYS).				
1	25	Range alone	None.	None.	None.	None.
2	25	Range plus half ration cotton-seed meal and hulls	236	854	2.41	8.71
3	25	Range plus half ration cotton seed	462	....	4.71	....
4[a]	25	Range plus cheap mixed hay	....	826	....	11.8

[a] This lot was fed only seventy days—January 1 to March 12.

During each year's work those steers that received feed in addition to the range were started on a very small daily allowance. This amount was increased every few days for twenty-eight days, when it was held uniform for the remainder of the winter.

There was no way to determine how much feed was secured from the range as far as weight was concerned. Each steer had about 10 acres as a winter range. The steers in lot 1, the range lot, had to be turned out upon the general range each winter about a month before the end of the test, as their range of 10 acres each had become exhausted about thirty days earlier than was the case with those lots which were receiving supplementary feeds.

During the first winter each steer in lot 2 consumed, in addition to the range, 197 pounds of cotton-seed meal and 714 pounds of hulls, while each steer the second winter ate 236 pounds of cotton-seed meal and 854 pounds of hulls. But the animals were fed fourteen days longer the second than the first winter. Each steer's daily ration was kept a little below 2.5 pounds of cotton-seed meal and from 8.5 to 8.71 pounds of hulls.

During the second winter the steers in lot 3 were carried through the winter on cotton seed as a supplement to the range. It was learned that 4.71 pounds of cotton seed per steer per day was not quite sufficient to keep the animals from losing weight. Each steer lost 39.6 pounds in weight during the winter period of ninety-eight days.

In the first winter's work it is seen that 8.5 pounds of good peavine hay, along with the range, afforded the steers sufficient daily feed to allow them to maintain a practically uniform weight. Or, when lots 2 and 3 (1907–8) are compared, it is seen that 714 pounds of cow-pea hay were practically equal in feeding value to 197 pounds of cotton-seed meal plus 714 pounds of cotton-seed hulls. In other words, the cow-pea hay was worth $13.02 per ton for wintering mature cattle compared with cotton-seed meal and hulls, when the meal is valued at $26 a ton and the hulls at $6 a ton.

During the second winter (1908–9) each steer in the cotton-seed lot (lot 3) lost about 43 pounds more in live weight than the animals in the cotton-seed meal and hulls lot (lot 2), but still, when cotton seed is valued at $14 a ton, it is probably cheaper than cotton-seed meal and hulls for wintering steers.

The daily expense of feeding each steer on cotton-seed meal and hulls was 5.7 cents, while the daily cost of the cotton seed alone per steer was only 3.3 cents. While not enough cotton seed was used to prevent loss in weight, still the amount fed daily to each steer (4.71 pounds) would probably not have to be increased very much to make the steers hold their fall weights. It would require 8.2 pounds of cotton seed, at $14 a ton, to cost as much as the 2.41

pounds of cotton-seed meal plus the 8.71 pounds of hulls which were fed to each steer daily in lot 2.

It should be remembered that these were mature steers, and that such steers are capable of making use of the rough waste feeds during the winter months. Cattle of this age can use feeds that would be entirely unsuited to young growing animals. In handling and feeding mature steers during the winter months the object should be to make use of all the rough feeds and unsalable hays before any high-priced feeds, as cotton-seed meal, are used.

GAINS AND LOSSES OF STEERS BY MONTHS.

Every southern farmer has old corn and cotton fields which afford some winter feeds for the cattle, but as the winter advances the range usually affords a smaller and smaller amount of feed. The following table shows the gain or loss of the various lots from month to month. From this the reader can gather some idea of when the heavy losses usually occur and regulate the amount of supplementary feeds accordingly.

TABLE 9.—*Results of winter feeding by months.*

WINTER OF 1907-8 (84 DAYS).

Lot.	Ration.	Gain (+) or loss (−) per steer, first month, December 10 to January 7.	Gain (+) or loss (−) per steer, second month, January 7 to February 4.	Gain (+) or loss (−) per steer, third month, February 4 to March 3.
		Pounds.	*Pounds.*	*Pounds.*
1	Range alone	−4	−38	−55
2	Range plus half ration cotton-seed meal and hulls	+16	−10	−12
3	Range plus half ration cow-pea hay	+15	−16	−8

WINTER OF 1908-9 (98 DAYS).

		December 4 to January 1.	January 1 to January 29.	January 29 to February 26.	February 26 to March 12.
1	Range alone	−40	−43	−7	−16
2	Range plus half ration cotton-seed meal and hulls.	+18	−12	−5	+2
3	Range plus half ration cotton seed	0	−16	−46	+22
4	Range plus cheap mixed hay		−13	−23	−4

In the first winter's work the range cattle (lot 1) practically held their initial weight during the first twenty-eight days. As time went on and the range became shorter and shorter they lost more and more in weight. This is what should be expected. But the heaviest losses in 1908-9 were experienced at the early part of the winter. However, this winter was an unusual one. It was very rainy and muddy during the early months, so that the cattle were very uncomfortable and could not graze well. During the last of the winter very little rain fell and spring set in early, so, as a matter of fact, the range cattle had some green feed during the last month in addition to the range. The table shows that all the lots except lot 1 showed considerable improvement in the last two weeks of the winter period of 1909.

As stated elsewhere, all of these cattle came through the winter in good grazing condition; they were strong and active, although the steers in the range lots (lot 1) had fallen off in live weight about 100 pounds each.

FINANCIAL STATEMENT FOR WINTER WORK.

In the fall of 1907 the steers cost $2.50 per hundredweight, but the next fall, 1908, feeders had advanced some in price, making the fall price average $2.56 per hundredweight. In the spring the cost was, of course, considerably greater than the fall price for two reasons: First, the cattle were not as heavy as they were the previous fall, and second, the cost of winter feed had to be added to the fall price. The fall cost, added to the depreciation in live weight, and the cost of winter feed made the steers cost about $3 per hundredweight in the spring. The following table, No. 4, shows the spring cost by lots. The average of these spring costs was taken as the initial cost of the steers in the summer feeding work which followed. The average cost of wintering each steer in the various lots, together with the difference in value between fall and spring, are as follows:

Table 10.—*Financial statement.*

1907–8.

Lot 1. **Range alone:**		
To 722-pound steer, at $2.50 per hundredweight	$18.05	
By value of same steer in spring, 625 pounds, at $2.89 per hundredweight		$18.05
	18.05	18.05
Lot 2. **Range plus cotton-seed meal and hulls:**		
To 726-pound steer, at $2.50 per hundredweight	18.15	
To 714 pounds cotton-seed hulls, at $6 per ton	2.14	
To 197 pounds cotton-seed meal, at $26 per ton	2.56	
By value steer in spring, 720.5 pounds, at $2.89 per hundredweight		20.82
By required increase in value over range steer to break even (28 cents per hundredweight)		2.03
	22.85	22.85

1907-8.

Lot 3. Range plus peavine hay:

To 724-pound steer, at $2.50 per hundredweight	$18.10	
To 714 pounds peavine hay, at $10 per ton	3.57	
By value steer in spring, 715 pounds, at $2.89 per hundredweight		$20.65
By required increase in value over range steer to break even (14 cents per hundredweight)		1.02
	21.67	21.67

1908-9.

Lot 1. Range alone:

To 705-pound steer, at $2.56 per hundredweight	18.05	
By value same steer in spring, 599 pounds, at $3.01 per hundredweight		18.05
	18.05	18.05

Lot 2. Range plus cotton-seed mealand hulls:

To 705-pound steer, at $2.56 per hundredweight	18.05	
To 854 pounds cotton-seed hulls, at $6 per ton	2.56	
To 236 pounds cotton-seed meal, at $26 per ton	3.07	
By value steer in spring, 708 pounds, at $3.01 per hundredweight		21.31
By required increase in value over range steer to break even (33 cents per hundredweight)		2.37
	23.68	23.68

Lot 3. Range plus cotton seed:

To 706-pound steer, at $2.56 per hundredweight	18.07	
To 462 pounds cotton seed, at $14 per ton	3.23	
By value steer in spring, 666 pounds, at $3.01 per hundredweight		20.05
By required increase in value over range steer to break even (19 cents per hundredweight)		1.25
	21.30	21.30

Lot 4. Range plus cheap hay:

To 703-pound steer, at $2.56 per hundredweight	18.00	
To 826 pounds waste hay, at $5 per ton	2.06	
By value steer in spring, 649 pounds, at $3.01 per hundredweight		19.53
By required increase in value over range steer to break even (8 cents per hundredweight)		.53
	20.06	20.06

The total cost to winter each steer in 1907-8 was $4.70 and $3.57 in lots 2 and 3, respectively. The range has no price placed upon it, although the results show that it has a very great value. The total cost to winter each steer in 1908-9 was $5.63, $3.23, and $2.06 in lots 2, 3, and 4, respectively.

After the cost of wintering the cattle and the winter shrinkage were added to the fall buying price the spring cost was obtained. The spring costs in lots 1, 2, and 3 in 1907-8 were $2.89, $3.17, and $3.03 per hundredweight, respectively. In 1908-9 the spring costs were $3.01, $3.34, $3.20, and $3.09 per hundredweight in lots 1, 2, 3, and 4, respectively.

It is seen that the cheap coarse feeds produced about as good results as the high-priced feeds, while the steers were carried through the winter much more economically with the cheap than with the expensive feeds. It will always pay to make use of the coarse or cheap winter feeds for the mature steers and save the high-priced feeds for the young animals of the farm.

It is well known that the effects of feeding mature cattle through the winter months continue throughout the following grazing season. Those mature cattle that experience the least loss in weight during the winter may be expected to make the smallest gains the following summer. This has been found to be true in this work, but a detailed presentation of this point will be found in a later publication.

TABLE 11.—*Total summary of winter work.*

Item.	1907-8 (84 days).			1908-9 (98 days).			
	Lot 1, range alone.	Lot 2, range and cotton-seed meal and hulls.	Lot 3, range and cowpea hay.	Lot 1, range alone.	Lot 2, range and cotton-seed meal and hulls.	Lot 3, range and cotton seed.	Lot 4, range and waste hay.
Average weight of steers at beginning of test..........pounds..	722	726	724	705	705	706	689
Average weight at end of test..........do....	625	720	715	599	708	666	649
Total gain (+) or loss (−) per steer for whole winter..........pounds..	−97.	− 6.	− 9.	−106	+ 3.	−40.	−40.
Average daily gain (+) or loss (−) per steer, pounds..........	− 1.15	− .07	− .11	− 1.08	+ .03	− .41	− .57
Concentrates consumed per steer per day, pounds..........	....	2.35	....	....	2.41	4.71	....
Roughage consumed per steer per day, pounds..........	....	8.5	8.5	....	8.71	....	11.8
Average expense to winter each steer..........	....	$4.70	$3.57	....	$5.63	$3.23	$2.06
Initial, or fall cost of steers per hundredweight.	$2.50	$2.50	$2.50	$2.56	$2.56	$2.56	$2.56
Total cost of steers per hundredweight following spring..........	$2.89	$3.17	$3.03	$3.01	$3.34	$3.20	$3.09

SUMMARY OF WINTER WORK.

1. The cattle used in all of these tests were practically mature ones.

2. Winter rations used:

	1907-8.	1908-9.
Lot 1..........	Range alone..........	Range alone.
Lot 2..........	Range plus half ration cotton-seed meal and hulls.	Range plus half ration cotton-seed meal and hulls.
Lot 3..........	Range plus half ration peavine hay....	Range plus half ration cotton seed.
Lot 4..........	....	Range plus half ration cheap hay.

3. In 1907–8 each range steer (lot 1) lost 97 pounds in weight. In 1908–9 each range steer (lot 1) lost 106 pounds in weight.

4. In 1907–8 each steer in lot 2 received 2.35 pounds of cotton-seed meal and 8.5 pounds of hulls each day in addition to the range.

During the winter of 1908–9 each steer in lot 2 received 2.41 pounds of cotton-seed meal and 8.71 pounds of hulls daily. The first year each steer lost 6 pounds in weight; the second year each steer gained 3 pounds in weight.

5. In 1907–8 each steer in lot 3 was fed a daily ration of 8.5 pounds of good cowpea hay in addition to the range; the loss in weight per steer for the winter was 9 pounds.

6. In 1908–9 cotton seed was tried as a supplement to the range, 4.71 pounds being fed to each steer daily. The loss in weight per steer for the winter was 40 pound.

7. In 1908–9 cheap hay was used in lot 4 to supplement the range, 11.8 pounds being fed to each steer daily. The winter loss per steer was 40 pounds.

8. The total cost to winter each steer in 1907–8 was $4.70 and $3.57 in lots 2 and 3, respectively. The total cost to winter each steer in 1908–9 was $5.63, $3.23, and $2.06 in lots 2, 3, and 4, respectively.

9. In 1907–8 the fall buying price was $2.50 per hundredweight. When the expense of wintering the steers was added to the fall price the spring prices were found to be $2.89, $3.17, and $3.03 per hundredweight in lots 1, 2, and 3, respectively.

10. In 1908–9 the fall buying price was $2.56 per hundredweight. When the expense of wintering the steers was added to the fall price the spring prices were found to be $3.01, $3.34, $3.20, and $3.09 per hundredweight in lots 1, 2, 3, and 4, respectively.

III. FATTENING CATTLE ON PASTURE.

INTRODUCTION.

As a rule the ordinary permanent pastures in Alabama can be depended upon to furnish grazing from about April 1 to some time in October. The frosts usually kill the pastures in October. By making use of winter-growing plants, such as bur clover, the grazing season can be opened about February 1, and sometimes even earlier.

A common mistake is to overstock the pastures. When this is done the grass often becomes short in August and September, and the cattle actually lose in weight instead of gaining. Furthermore, the South often experiences a drought in August and September, therefore the farmer should have no more cattle on hand than can be well cared for during the grazing period.

The pastures used in this test, as stated before, were made up of several kinds of grasses. No one kind of plant was depended upon entirely. Johnson grass, Japan clover, and sweet clover were the most important grazing plants used. In addition to these some Bermuda and crab grasses were also found. If the pastures are to be improved year by year and the grazing season extended over as many months as possible, several plants must be made use of.

The cattle used in the summer feeding work were the same as had been used in the preceding winter's experimental work, except that some steers which had not been in the winter experiment were added to the summer work. These extra steers had been fed nothing through the winter months except what they obtained on the open range. They were of the same quality as the steers which had been in the winter tests. All of these cattle had been dehorned the previous winter. There were, in addition, a mixed lot of scrub steers (lot D), which are described later on. When grass appeared in the spring the winter work was discontinued, the cattle were redivided into lots, and the summer feeding work was begun immediately.

THE SUMMER PASTURE.

The summer pasture used in these experiments consisted of a mixture of sweet clover (Melilotus), Japan clover (Lespedeza), Johnson grass, crab grass, and some Bermuda grass. The sweet clover became available for grazing about April 1, while the Japan clover was not ready until about June 15. In some sections of the country sweet clover is considered a pest, as stock will not eat it, but in the South, or at least in Alabama, all kinds of stock eat it with great relish; they take to it as readily as to alfalfa.

The pasture was divided into lots, the size of each lot depending upon the number of cattle grazed upon it, and as to whether the steers were to be fed a concentrated supplement or not. The object was to have an abundance of pasture for each bunch of cattle. The method of feeding and handling the cattle has already been described in connection with the winter work.

DETAILS OF THE SUMMER FEEDING.

FEEDS CONSUMED.

The cattle were fed but once a day; this was done each afternoon about sundown, or in the cool of the evening, in order that the steers would all come out to the feed troughs. The steers were started upon a small amount of feed, and as they became accustomed to it the amount was gradually increased. The following tables will show that the concentrates were fed sparingly all through the tests.

Table 12 shows the total amount of concentrates fed each steer for each year, the average daily feed, and the amount of concentrates required to make 100 pounds of gain.

TABLE 12.—*Feeds consumed.*

1908 (112 DAYS).

Lot.	Number cattle in lot.	Ration.	Total amount feed consumed per steer.	Amount feed consumed per steer per day.	Pounds feed to make 100 pounds gain.
			Pounds.	*Pounds.*	*Pounds.*
A	26	Pasture alone			
B	26	Pasture plus cotton-seed cake	371	3.31	143
C	26	Pasture plus cold-pressed cotton-seed cake	371	3.31	180
D[a]	54	Pasture plus cotton-seed cake	309	2.76	171

1909 (154 DAYS).

Lot.	Number cattle in lot.	Ration.	Total amount feed consumed per steer.	Amount feed consumed per steer per day.	Pounds feed to make 100 pounds gain.
A	40	Pasture alone			
B	75	Pasture plus cotton-seed cake	524	3.40	181
E	25	Pasture plus cotton seed	691	4.49	218

[a] The cattle in lot D were not of the same grade as those in the other lots and are hardly comparable with them. Lot D was made up of a bunch of cattle with no special breeding, and they ranged from 2 to 5 years in age. The object in handling this lot was to see if money could be made on such cattle. They had not been dehorned.

Lot A received no feed in addition to the pasture as one object was to learn whether it would pay to supplement the pasture with a concentrate. During the summer of 1908 each steer in lot B was given 3.31 pounds of cotton-seed cake in addition to the pasture; in 1909 each steer in this lot was fed 3.4 pounds of the cake in addition to the pasture. In 1908 the cold-pressed cotton-seed cake was used with one lot so that its value as a feed could be compared with ordinary cotton-seed cake. The steers in lot D (the mixed lot) in 1908, on account of being smaller than the ones in the other lots, were fed only 2.76 pounds of cotton-seed cake per head per day. In 1909 cotton seed was fed to lot E.

When looking at the last column it is seen that the cotton-seed cake was more efficient than the cold-pressed cake for making gains. In 1908 only 143 pounds of the former were required to make 100 pounds gain, while 180 pounds of the latter were required to make the same number of pounds gain (lot D is not used in this comparison for reasons previously stated). It is true that the cold-pressed cake did not cost as much as the regular cotton-seed cake, but it will be seen later, when the cost of the feeds are taken into consideration, that the cotton-seed cake was the more economical feed to use. Under the conditions of this test 1 pound of cotton-seed cake was equal in feeding value to 1.26 pounds of the cold-pressed cake.

During the summer of 1909 a direct comparison was made between cotton-seed cake and cotton seed as feeds to be used to supplement pastures. Under the conditions of this test 1 pound of cake proved to be equal to 1.20 pounds of the seed. The seed proved to have an exceedingly high feeding value when used as a feed to supplement the pastures.

The steers in lot D were a bunch of mixed scrubs, varying from 2 to 5 years in age. There was very little improved blood among these cattle. They were not dehorned, so they were always restless at the feed trough, as the timid ones were afraid of the steers with long sharp horns. This lot was fed as a side issue to the main experiment to determine whether a profit could be made upon this class of cattle.

There was no way, of course, to determine just how much pasture grass was consumed, except as to the area measured off for each lot. But it is interesting to note that the amount of concentrated feeds required to make 100 pounds increase in live weight was exceedingly small. This was due to at least two factors. First, the steers had a green feed to go along with the concentrated feeds. Second, the amount of concentrated feeds fed daily was held down to a few pounds, thus requiring the steers to obtain the major part of their feed from the pasture. Where lands are cheap pasture is cheaper than the too liberal use of concentrated feeds. It is impossible, at the present time, to say whether the amounts fed in these tests were the correct ones or not, but it is hoped that some light may be thrown upon this point, during the further progress of the work.

GAINS MADE.

The gains as recorded in the following table will show that the pastures used were good ones. It should be remembered, too, that as a result of feeding the cattle upon these pastures the latter are getting better and better as time goes on. The table sets forth the total and daily gains of the steers during the summers of 1908 and 1909.

TABLE 13.—*Total and daily gains during the pasture-feeding test.*

1908 (112 DAYS).

Lot.	No. of steers in lot.	Ration.	Average initial weight per steer.	Average final weight per steer.	Total gain per steer.	Average daily gain per steer.
			Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
A	26	Pasture alone	732	902	170	1.52
B	26	Pasture plus cotton-seed cake	739	999	260	2.32
C	26	Pasture plus cold-pressed cotton-seed cake	738	944	206	1.84
D	54	Pasture plus cotton-seed cake	532	713	181	1.62

1909 (154 DAYS).

Lot.	No. of steers in lot.	Ration.	Average initial weight per steer.	Average final weight per steer.	Total gain per steer.	Average daily gain per steer.
A	40	Pasture alone	647	915	268	1.74
B	75	Pasture plus cotton-seed cake	639	929	290	1.88
E	25	Pasture plus cotton seed	653	970	317	2.06

It is seen that in every case those cattle that received some supplementary feed gained more rapidly than those which received no feed but pasture. Of course, the more rapidly a steer gains the quicker he can be gotten in shape for the market, and this is a very important point, as the early fall steer does not come into competition with the fall stock that is being brought into the market off grass.

As far as gains were concerned, the cattle did reasonably well both years. They were not grazed through the whole summer season, so the total gains, as represented in the sixth column, do not represent as great gains as can be made during a whole summer's grazing season. In 1908 they were grazed only one hundred and twelve days, and in 1909 the test continued for one hundred and fifty-four days. The best portion of the grazing season had been used, however, as the cattle were sold in the first part of September.

In both years the cattle which received cotton-seed cake in addition to the pasture gained more rapidly than the pasture cattle. In 1908 the difference in favor of the cattle which had been fed the cake was very marked, but in 1909 the difference between the two lots was not very pronounced. In 1909 lot E, the cotton-seed lot, made the most satisfactory gains, they making an average daily gain of 2.06 pounds, while lots B and A made average daily gains of 1.88 and 1.74 pounds, respectively.

COST OF GAINS.

It is always unsatisfactory to discuss the cost of gains, as this depends largely upon the cost of the feeds, which varies greatly under different conditions. In this discussion the price placed upon the feeds is the actual market quotation plus the expense of hauling them from the depot to the farm. The hauling distance was 15 miles. Pasture is charged at 50 cents per month per steer; this is the prevailing price placed upon pastures throughout the western part of Alabama.

TABLE 14.—*Cost to make 100 pounds of gain.*

1908 (112 DAYS).

Lot.	Ration.[a]	Pounds feed to make 100 pounds gain.	Cost 100 pounds gain, pasture not charged.	Cost 100 pounds gain, pasture charged.
A	Pasture alone			$1.18
B	Pasture plus cotton-seed cake	143	$1.79	2.56
C	Pasture plus cold-pressed cotton-seed cake	180	2.07	3.04
D	Pasture plus cotton-seed cake	171	2.14	3.04

1909 (154 DAYS).

Lot.	Ration.[a]	Pounds feed to make 100 pounds gain.	Cost 100 pounds gain, pasture not charged.	Cost 100 pounds gain, pasture charged.
A	Pasture alone			$1.03
B	Pasture plus cotton-seed cake	181	$2.26	3.21
E	Pasture plus cotton-seed	218	1.53	2.39

[a] Price of feeds: Cotton-seed cake, $25 per ton; cold-pressed cotton-seed cake, $23 per ton; cotton seed, $14 per ton; pasture, 50 cents per month.

In every case above the cost to make 100 pounds increase in live weight was very low. When steers are fattened during the winter time each pound of gain is put on at a loss, as each pound put on may be expected to cost from 8 to 12 cents, and the profit is, of course, dependent upon the enhancement of the value of the steer over and above the selling value of pounds of gain made. In these tests each pound put on during the fattening period was put on at a profit. These cheap finishing gains made the feeding operations comparatively safe as far as profits were concerned. As stated before, these cheap gains were due to two factors. First, the cattle had a cheap and succulent roughage—pasture. Second, the amount of concentrated feeds used was kept down to a comparatively small figure; from 2.76 to 3.31 pounds of cotton-seed cake and 4.49 pounds of cotton-seed were fed to each steer daily. At the Missouri station (see Bulletin 76) the average of the summer trials shows that 814 pounds of grain were required to produce 100 pounds of gain, while in our test only 143 to 218 pounds of concentrate were required to make the same gains. At Missouri the steers were given an approximate daily feed of 20 pounds of grain in addition to the pasture. It may be mentioned, also, that while the Missouri cattle were fed a much heavier grain ration than ours, the records show that the Alabama cattle made almost as large daily gains as the Missouri steers.

When lots B and C (1908) are compared it is seen that the cotton-seed cake is superior to the cold-pressed cake, as 100 pounds of increase in weight were made at a cost of $2.56 when the former cake was used, whereas when the latter cake was fed the same gains cost $3.04. When regular cotton-seed cake sells at $25 a ton the cold-pressed cake is not worth $23 a ton. When cotton-seed cake sells at $25 a ton this test shows the cold-pressed cake containing the hulls to be worth $19.83 a ton on a comparative basis.

The common or mixed bunch of cattle (lot D) make a very poor showing when compared with lots B and C, although, as will be seen later, the steers in lot D returned a small profit.

In comparing lots B and E (1909) it is seen that the cotton seed produced gains more cheaply than did the cotton-seed cake; that is, when the seed is valued at $14 a ton and the cake at $25 a ton. When cotton-seed cake is valued at $25 a ton, this test shows the cotton seed to be worth $20.76 a ton for fattening cattle on pasture. Cotton seed has this disadvantage, however, that during the latter part of the feeding period it is not relished as much as the cake, and some trouble was experienced in keeping the steers "on feed." There was no trouble from scouring when the seed was fed in the above amounts.

FINANCIAL RESULTS OF SUMMER FEEDING.

Although the cattle that received pasture alone made cheaper gains than those that received some feed in addition to the pasture, it must not be inferred that the grass cattle were the most profitable ones; the cost of the gains alone does not determine the final profits. While it is desirable to make the gains as cheaply as possible, still the selling price of the cattle at the end of the feeding period must also be taken into consideration before the final profit can be determined.

TABLE 15.—*Financial statement.*

1908.

Lot A. Pasture alone:		
To 26 steers, 19,031 pounds, at $2.92 per hundredweight	$555.71	
To pasture, at 50 cents a month per steer	52.00	
To freight, commission, feed, and yardage	94.12	
Total expenditures	701.83	
By sale of 26 steers, 21,210 pounds, at $3.66 per hundredweight		$776.29
Total profit on lot	74.46	
Profit per steer	2.86	

1908.

Lot B. Pasture plus cotton-seed cake:		
To 26 steers, 19,199 pounds, at $2.92 per hundredweight	$560.61	
To pasture, at 50 cents a month per steer	52.00	
To 9,646 pounds of cotton-seed cake, at $25 per ton	120.57	
To freight, commission, feed and yardage	94.12	
Total expenditures	827.30	
By sale of 26 steers, 24,245 pounds, at $4.53 per hundredweight		$1,098.30
Total profit on lot	271.00	
Profit per steer	10.42	
Lot C. Pasture plus cold-pressed cotton-seed cake:		
To 26 steers, 19,176 pounds, at $2.92 per hundredweight	559.94	
To pasture, at 50 cents a month per steer	52.00	
To 9,646 pounds of cake, at $23 per ton	110.93	
To freight, commission, feed, and yardage	94.12	
Total expenditures	816.99	
By sale of 26 steers, 22,740 pounds, at $4.35 per hundredweight		989.19
Total profit on lot	172.20	
Profit per steer	6.62	
Lot D. Pasture plus cotton-seed cake:		
To 54 steers, 28,754 pounds, at $2.50 per hundredweight	718.85	
To pasture, at 50 cents a month per steer	108.00	
To 16,686 pounds of cotton-seed cake, at $25 per ton	208.57	
To freight, commission, feed, and yardage	195.48	
Total expenditures	1,230.90	
By sale of 54 steers, 36,450 pounds, at $3.44 per hundredweight		1,253.88
Total profit on lot	22.98	
Profit per steer	.43	

1909.

Lot A. Pasture alone:		
To 40 steers, 25,879 pounds, at $2.95 per hundredweight	763.43	
To pasture, at 50 cents a month per steer	110.00	
To freight, commission, feed, and yardage	144.80	
Total expenditures	1,018.23	
By sale of 40 steers, 34,314 pounds, at $3.79 per hundredweight		1,300.50
Total profit on lot	282.27	
Profit per steer	7.06	
Lot B. Pasture plus cotton-seed cake:		
To 75 steers, 47,916 pounds, at $2.95 per hundredweight	1,413.52	
To pasture, at 50 cents a month per steer	206.25	
To 39,325 pounds of cotton-seed cake, at $25 per ton	491.56	
To freight, commission, feed, and yardage	271.50	
Total expenditures	2,382.83	

1909.

Lot B. Pasture plus cotton-seed cake—Continued.		
By sale of 75 steers, 66,514 pounds, at $4.37 per hundredweight		$2,906.66
Total profit on lot	$523.83	
Profit per steer	6.99	
Lot E. Pasture plus cotton seed:		
To 25 steers, 16,328 pounds, at $2.95 per hundredweight	481.68	
To pasture, at 50 cents a month per steer	68.75	
To 17,265 pounds of cotton seed, at $14 per ton	120.85	
To freight, commission, feed, and yardage	90.50	
Total expenditures	761.78	
By sale of 25 steers, 22,858 pounds, at $4.25 per hundredweight		971.46
Total profit on lot	209.68	
Profit per steer	8.39	

It should be noted that the total profits shown above are based on estimates after pasture rent, freight, commission, feed, etc., are taken from the total sales.

In 1908 it cost $3.85 per head to get the steers to the New Orleans market, and in 1909 the expense was $3.62 per head. These cattle were shipped from western Alabama to New Orleans, a distance of about 500 miles; many farmers in the South are not required to ship their cattle this distance.

In 1908 the greatest profits were realized upon lot B, the lot which received cotton-seed cake in addition to the pasture; in this lot a net profit of $10.42 per steer was made. Lot C, the lot fed on cold-pressed cake, returned a net profit of $6.62 per steer. The pasture lot, lot A, made a profit of only $2.86 per steer. It paid to feed the cattle some feed in addition to the pasture, because when offered for sale the steers thus fed were in much better condition than those that received pasture only, and consequently sold for more money per hundredweight. The grass cattle sold for $3.66 per hundredweight, the cotton-seed cake cattle for $4.53 per hundredweight, and the cattle fed on cold-pressed cake for $4.35 per hundredweight. The above represent the New Orleans prices. It cost about 60 cents per hundredweight, including shrinkage, to ship the steers to New Orleans.

In 1909 there was not a very great difference in favor of the lots which received feed in addition to the pasture. In fact, the pasture lot, lot A, and the cotton-seed cake lot (lot B) made practically the same profit, the former making a net profit of $7.06 per steer and the latter a net profit of $6.99 per steer. But the cotton-seed lot (lot E) was more profitable than either of the others, making a net profit of $8.39 per steer. The profits in every case were exceedingly satisfactory.

In 1908 it proved to be exceedingly profitable to supplement the pasture with a concentrated feed. In 1909 practically no extra profit was made as a result of the use of cotton-seed cake, but when cotton seed was fed along with the pasture the profits were greater than when pasture was used alone. The data so far collected warrant the statement that it pays to supplement the southern pastures with a concentrated feed when cattle are being finished for the fall market. Additional experimental work will determine what concentrated feeds can be used to the greatest advantage.

SLAUGHTER RESULTS.

The cattle were shipped to New Orleans for sale and were there slaughtered. Complete individual slaughter records were secured from the abattoir. Some of the details are presented in the table below, the remainder being reserved for a fuller discussion in a subsequent publication:

TABLE 16.—*Slaughter data.*

1908.

Lot.	Ration.	Average farm weight of steers.	Average New Orleans weight of steers.	Average shrinkage in shipping.	Percentage dressed of farm weight.	Percentage dressed of New Orleans weight.
		Pounds.	*Pounds.*	*Pounds.*		
A	Pasture alone	902	816	86	49.5	53.1
B	Pasture plus cotton-seed cake	999	933	66	53.8	55.9
C	Pasture plus cold-pressed cotton-seed cake	944	874	70	53.6	56.1
D[a]	Pasture plus cotton-seed cake	724	686	38	52.7	53.8
	1909.					
A[b]	Pasture alone	916	859	57	51.8	53.6
B[c]	Pasture plus cotton-seed cake	941	899	42	54.2	55.0
E[d]	Pasture plus cotton seed	1,001	946	55	53.9	55.3

[a] Slaughter data secured on 45 head only, figures on basis of those.
[b] These figures based on the slaughter data of 28 head at New Orleans.
[c] These figures based on the slaughter data of 48 head at New Orleans.
[d] These figures based on the slaughter data of 19 head at New Orleans.

It is seen by the above table that the steers which were given some feed in addition to the pasture suffered less loss from shipping than the ones which had nothing to eat but pasture. In 1908 each pasture steer (lot A) lost 86 pounds in transit, while those which had been fed some concentrated feeds averaged from 38 to 70 pounds for each lot. The common cattle, lot D, suffered a very small shrinkage, which was due, in part, to their being smaller steers than the other cattle. The cattle did not shrink as much in 1909 as in 1908, but in 1909 the grass cattle lost considerably more weight in transit than did those that had been fed.

In both years the steers which had been fed the cotton-seed products dressed out several per cent higher than the grass cattle. In 1908 the grass cattle dressed out 49.5 per cent; in 1909 a similar lot dressed out 51.8 per cent. The cattle which had been given some concentrated feeds along with the pasture dressed about 54 per cent. It should be noted that the next to the last column is based on the farm weights of the cattle. When the New Orleans live weights are taken, as in the last column, it raises the figures in the last column from 2 to 2.5 points in each case.

TABLE 17.—*Total summary of summer feeding.*

	1908 (112 days).				1909 (154 days).		
	Lot A, pasture alone.	Lot B, pasture plus cotton-seed cake.	Lot C, pasture plus cold-pressed cotton-seed cake.	Lot D, pasture plus cotton-seed cake.	Lot A, pasture alone.	Lot B, pasture plus cotton-seed cake.	Lot E, pasture plus cotton seed.
Average weight at beginning of test..................pounds..	732	739	738	532	647	639	653
Average weight at end of test, pounds..........................	902	999	944	713	915	929	970
Average daily gain....pounds..	1.52	2.32	1.84	1.62	1.74	1.88	2.06
Average amount concentrates consumed per day...pounds..	....	3.31	3.31	2.76	....	3.40	4.49
Average amount concentrates to make 100 pounds gain, pounds...........................	....	143	180	171	....	181	218
Cost 100 pounds gain, pasture charged...........................	$1.18	$2.56	$3.04	$3.24	$1.03	$3.21	$2.39
Initial cost of steers per 100 pounds...........................	$2.92	$2.92	$2.92	$2.50	$2.95	$2.95	$2.95
Selling price per 100 pounds at New Orleans.................	$3.66	$4.53	$4.35	$3.44	$3.79	$4.37	$4.25
Total profit per steer...........	$2.86	$10.42	$6.62	$0.43	$7.06	$6.99	$8.39

SUMMARY OF SUMMER WORK.

1. The steers which were used in the winter work were redivided into lots and continued into the summer feeding work.

2. In 1908 the steers were fed a period of 112 days on pasture. In 1909 they were fed for 154 days.

3. The summer rations were:

	1908.	1909.
Lot A..............	Pasture alone..........................	Pasture alone.
Lot B..............	Pasture plus cotton-seed cake.........	Pasture plus cotton-seed cake.
Lot C..............	Pasture plus cold-pressed cotton-seed cake.	
Lot D..............	Pasture plus cotton-seed cake.........	
Lot E..............	....	Pasture plus cotton seed.

4. In 1908 the amount of feed used daily per steer, in addition to the pasture, was 3.31 pounds, 3.31 pounds, and 2.76 pounds in lots B, C, and D, respectively. In 1909 the daily amount of feed used per steer to supplement the pasture was 3.4 pounds and 4.49 pounds in lots B and E, respectively.

5. In 1908 the average daily gains were 1.52, 2.32, 1.84, and 1.62 pounds in lots A, B, C, and D, respectively. In 1909 the average daily gains were 1.74, 1.88, and 2.06 pounds in lots A, B, and E, respectively.

6. In 1908 the total cost to make 100 pounds of gain was $1.18, $2.56, $3.04, and $3.24 in lots A, B, C, and D, respectively. In 1909 the total cost to make 100 pounds of gain was $1.03, $3.21, and $2.39 in lots A, B, and E, respectively.

7. In 1908 the net profits per steer were $2.86, $10.42, $6.62, and $0.43 in lots A, B, C, and D, respectively. In 1909 the net profits per steer were $7.06, $6.99, and $8.39 in lots A, B, and E, respectively.

8. In 1908 the steers dressed out (farm weights) 49.5 per cent, 53.8 per cent, 53.6 per cent, and 52.7 per cent in lots A, B, C, and D, respectively. In 1909 they dressed out (farm weights) 51.8 per cent, 54.2 per cent, and 53.9 per cent in lots A, B, and E, respectively.

9. These experiments are being continued.

Issued March 17, 1911.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 132.
A. D. MELVIN, CHIEF OF BUREAU.

A BACTERIOLOGICAL STUDY OF HAM SOURING.

BY

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WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

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LETTER OF TRANSMITTAL.

United States Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., November 2, 1910.

Sir: I have the honor to transmit and to recommend for publication as a bulletin of this Bureau a paper entitled "A Bacteriological Study of Ham Souring," by Dr. C. N. McBryde, senior bacteriologist in the Biochemic Division of this Bureau.

The souring of hams is a source of considerable loss in the meat-packing industry, and the cause of this trouble has heretofore been in doubt. Dr. McBryde's paper presents the results of an exhaustive study of the subject, from which it appears that he has succeeded in discovering the true cause of the trouble. Besides a description of the experimental work the paper discusses methods of preventing the souring of hams and the proper disposal of those which have become affected.

Respectfully,
A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.
Introductory 7
Method of curing hams 8
Definition of souring 10
Classification of sour hams and location of sour areas 10
Method of detecting sour hams 12
Theories in regard to ham souring 12
Previous experimental work to determine cause of ham souring 13
The present experiments 14
  Media employed 14
  Method of procedure in examining hams 15
  Results of examination of sour and sound hams 16
  Histological changes in sour hams 17
  Chemical analyses of sour and sound hams 18
  Bacteriological examination of sour and sound hams 20
  Inoculation experiments with hams 21
  Probable method by which ham-souring bacillus enters hams 33
    Possibility of infection prior to slaughter 33
    Possible infection from pickling fluids 34
      Experiment to show whether infection takes place from the curing pickle 34
    Possible infection through manipulation or handling 35
      Infection from ham thermometers 35
      Experiment to show whether hams become infected from ham thermometers 37
      Infection from pumping needles 41
      Infection from billhooks 42
  Biological and morphological characteristics of the ham-souring bacillus 43
    Conditions favorable to growth 43
    Growth on different culture media 43
    Morphology 46
    Spore formation 46
    Resistance to heat and chemical agents 47
    Gas production 47
    Acid production 48
    Pathogenic properties 48
    Nature of the bacillus 48
Prevention of ham souring 50
General summary and conclusions 53
Acknowledgments 55

ILLUSTRATIONS.

PLATES.

Page.

Plate I. Fig. 1.—Section of muscular tissue from sound ham, showing muscle fibers cut longitudinally; nuclei sharply defined and cross striation distinct. Fig. 2.—Section of muscular tissue from sour ham, showing muscle fibers cut longitudinally; nuclei undergoing disintegration and cross striation indistinct........................ 16

II. Fig. 1.—Section through muscular tissue of ham which has undergone natural or spontaneous souring, showing distribution of bacilli between the muscle fibers, which are cut obliquely. Fig. 2.—Section through muscular tissue of ham which has undergone natural or spontaneous souring, showing individual bacilli between the muscle fibers, which are cut somewhat obliquely............ 16

III. Section through muscular tissue of artificially soured ham, showing distribution of bacilli between the muscle fibers, which are shown in cross section. Fig. 2.—Section through muscular tissue of artificially soured ham, showing individual bacilli between the muscle fibers, which are cut longitudinally...................... 26

IV. Glucose bouillon culture in Smith fermentation tube at four days.. 48

TEXT FIGURES.

Fig. 1. Cross section through body of ham, with sour areas indicated by shading and dotted lines.. 11

2. Cross section through body of ham to show method of sampling for chemical analysis.. 18

3. Cross section through body of artificially soured ham, showing sour areas and points at which cultures were taken.................. 25

4. Diagrammatic views showing construction of ham thermometer........ 36

5. Ham-souring bacillus (*Bacillus putrefaciens*), grown on egg-pork medium.. 46

A BACTERIOLOGICAL STUDY OF HAM SOURING.

INTRODUCTORY.

The souring of hams is a matter of considerable importance to those engaged in the meat-packing industry, and has been the occasion of no little worry, as in even the best-regulated packing establishments the yearly losses it entails are considerable. The subject has given rise to much speculation on the part of those engaged in the curing of meats, as to the cause of the trouble and how it may be remedied, and has received considerable attention in a practical way, but little seems to have been done in a scientific way toward determining the cause and nature of ham souring.

In a well-regulated meat-packing establishment the loss from ham souring is usually figured at about one-tenth of 1 per cent of the total weight of hams cured. At first thought this would seem but a small loss, but when one reflects that in a single large packing establishment some 3,000,000 hams are cured during the year, the loss, when figured out, is considerable. Taking 15 pounds as the average weight of a ham, 3,000,000 hams would represent 45,000,000 pounds of meat. Figuring the loss from souring on the basis mentioned, the amount of meat condemned and destroyed during the year would be 45,000 pounds. Assuming that hams sell at an average wholesale price of 15 cents a pound, the yearly loss for a single plant which cures 3,000,000 hams a year would be nearly $7,000.

While one-tenth of 1 per cent of the total weight of hams cured would represent the loss from souring in a well-regulated establishment, statistics obtained through Government meat inspectors show that 0.25 per cent would more nearly represent the loss for the entire country. During the fiscal year from July 1, 1908, to June 30, 1909, some 670,000,000 pounds of hams were placed in cure in packing establishments subject to Government inspection. Estimating the loss from souring at 0.25 per cent, the total amount of meat condemned and destroyed as sour would be 1,675,000 pounds. At 15 cents a pound the total annual loss from ham souring in packing houses subject to Government inspection would figure up something over a quarter of a million of dollars.

The problem of ham souring, therefore, is quite an important one from a practical and financial standpoint; but aside from these considerations it is also a subject of considerable scientific interest, and in view of the fact that all sour meats are condemned under the Federal regulations governing meat inspection it has seemed fitting that this question should be made the subject of scientific investigation on the part of the Bureau which is charged with the administration of this inspection.

The investigation reported in this paper has been conducted chiefly along bacteriological lines, and has been confined entirely to the wet method of curing hams, as this method is the one generally used in American packing houses.

METHOD OF CURING HAMS.

In order to make clear certain points in regard to the nature and occurrence of ham souring and to insure a better understanding of the experiments which are to be described later, it would seem best to begin with a brief outline of the method of curing hams as practiced in the larger packing establishments of the country. This description is merely a general outline of the method of preparing hams for cure and the method of handling hams while in cure, and deals chiefly with those points that bear on the question of souring.

After the slaughtered animal has been cleaned, scraped, eviscerated, washed, and split down the middle, the carcass is usually allowed to hang for an hour or so in a large room open to the outside air, known as the "hanging floor." This is done with a view to getting rid of a certain amount of the body heat before the carcass is run into the chill rooms, and effects a saving in refrigeration.

The carcasses are next run into "coolers" or chill rooms, and subjected to refrigeration with a view to ridding them entirely of their body heat. The coolers are large rooms fitted with brine pipes and capable of accommodating several hundred carcasses. The temperature of the coolers when the carcasses are run in is about 32° F. When filled, the temperature of the cooler rises to about 45° F., owing to the heat given off from the carcasses. The temperature is then gradually reduced to 28 or 30° F. Hog carcasses are left in the coolers as a rule for forty-eight hours, at the end of which time they are stiff and firm, but not frozen. The temperature of the chill rooms is always carefully watched, thermometer readings being made every few hours and duly recorded. The temperature of the carcasses is always tested when they leave the chill room. In those plants provided with a hanging floor, a certain number of the carcasses are also tested before they are sent to the chill rooms in order to determine the amount of heat lost on the hanging floor.

The carcasses are tested by means of an especially constructed thermometer, known as a "ham thermometer," which has a pointed metal protector so that it can be thrust into the body of the ham. (See fig. 4.) The ham has been rightly selected as the proper portion of the carcass at which to take the temperature, as it constitutes the largest mass of muscular tissue in the carcass and holds the body heat longer than any other portion. In taking the temperature, the thermometer is thrust deep into the body of the ham so that the point of the thermometer rests alongside or a little behind the upper portion of the femur or middle bone, the latter being used as a guide in introducing the thermometer. A certain number of the carcasses from each cooler are tested in this way as a check on the refrigeration. The inside temperature of the hams when they leave the chill rooms should be about 34° F.

The carcasses are next cut up and the hams trimmed for pickling. In some houses the hams are given an additional chilling of 48 hours after they are cut from the carcasses, but this is not done as a rule, nor does it seem to be necessary.

The hams are now sent to the pickling rooms, or "sweet pickle department," as this branch of the packing house is designated, and here a certain number are again tested with a thermometer, as described above. This test is carried out by the foreman in charge of the sweet pickle department in order that he may satisfy himself that the hams are properly chilled before they go into the pickle and as an additional check on the refrigeration.

The hams are now ready to be "pumped," and this pumping, as will be shown later, constitutes an important step in a successful cure. Pumping consists in forcing a strong brine solution containing saltpeter into the muscular tissues of the ham, and is accomplished by means of a large, hollow, fenestrated needle connected by means of a rubber hose with a powerful hand pump. The needle is introduced along the bone, the latter being used as a guide.

In all of the larger packing establishments two general methods of curing hams are followed, the two methods being designated as the "fancy" or "mild cure" and the "regular cure," the term "cure" being used to designate the curing period. Various trade names are given by the different packing establishments to the hams cured by these methods. In the fancy cure the hams are pumped in the shank only, whereas in the regular cure they are pumped in both body and shank. The same pumping pickle is generally used for the two cures. It is a significant fact that the greater proportion of the "sours" are found among the fancy or mild cure hams. This point will be discussed farther on in connection with some experiments to be described later.

The actual curing is usually carried out in large vats which hold about 1,400 pounds of meat or some hundred hams. The hams are packed in the vats in layers and are entirely covered with the pickling solution or brine. A certain proportion is always observed between the weight of the meat and the amount of the solution. The pickling solution, or "pickle," as it is termed, is a brine solution containing saltpeter and sugar. The composition of the pickle varies somewhat with the different packing establishments. The fancy-cure hams are usually cured in a milder pickle, that is, one that contains less salt and saltpeter than the pickle used in the regular cure, although in some packing establishments the same curing pickle is used for the two cures, the only difference being the additional pumping given the regular-cure hams. The pickling rooms, or "cellars," as they are called, are held at a temperature of 34° to 36° F., and the pickling solutions are always chilled to this temperature before being used.

The hams are allowed to remain in cure for about 60 days, and during this time are "overhauled" several times. Overhauling consists in throwing the hams from the vat in which they are packed into a neighboring empty vat, and then transferring the pickle to the new vat. The pickle is not changed, and the same pickle follows the hams through the entire curing process. The object in overhauling is to stir up the pickle and expose fresh surfaces of the meat to its action.

Hams are also cured in tierces which hold about 300 pounds of meat. In the tierce cure, the hams are packed in the tierces, the latter are then headed up, the pickling solution is next run in through the bunghole, so as to fill the tierce entirely, and a wooden stopper is finally driven into the bunghole. The tierces are rolled back and forth across the floor on dates corresponding to the dates of overhauling in the vat cure. The object of the rolling is to stir up the pickle, and in this way it corresponds to overhauling in the vat cure.

DEFINITION OF SOURING.

To the meat inspector, a sour ham is one which has a tainted or "off" odor, that is, any odor which deviates from the normal. The odor may be very slight, so slight that at times only the trained meat inspector can detect it. When slight, the odor is elusive and hard to define, but when pronounced it has a distinctly putrefactive quality. When not very pronounced, the odor possesses, as a rule, a slightly sour quality, chemically speaking, and at times this sour quality may be quite marked; hence the term "sour ham," or "sour" has originated. In a badly soured ham—using the term "sour" in the packing-house sense to denote any ham that is tainted—the odor loses this sour quality and becomes distinctly putrefactive in nature.

CLASSIFICATION OF SOUR HAMS AND LOCATION OF SOUR AREAS.

Sour hams are classed as "shank sours" and "body sours," according to the location of the souring, and these may be "light" or "heavy." When the souring is very pronounced, the ham is termed a "stinker."

Souring appears to start, as a rule, around the stifle joint (femorotibial articulation), and extends upward into the body of the ham.

In quite a large proportion of the hams which are sour in the body—probably from 40 to 50 per cent—the souring extends through to the bone marrow of the femur or middle bone, and the sour odor is at times more pronounced in the bone marrow than in the meat. The odor of the bone marrow, when pronounced, is strongly suggestive of a dissecting-room odor, and is distinctly putrefactive in quality.

In the case of light body sours the sour odor is confined to a small area immediately around the bone, and may be so slight that it is detected only with difficulty. In such hams the bone marrow is apt to be sweet, and it is not until the souring becomes more extensive that the bone marrow becomes involved.

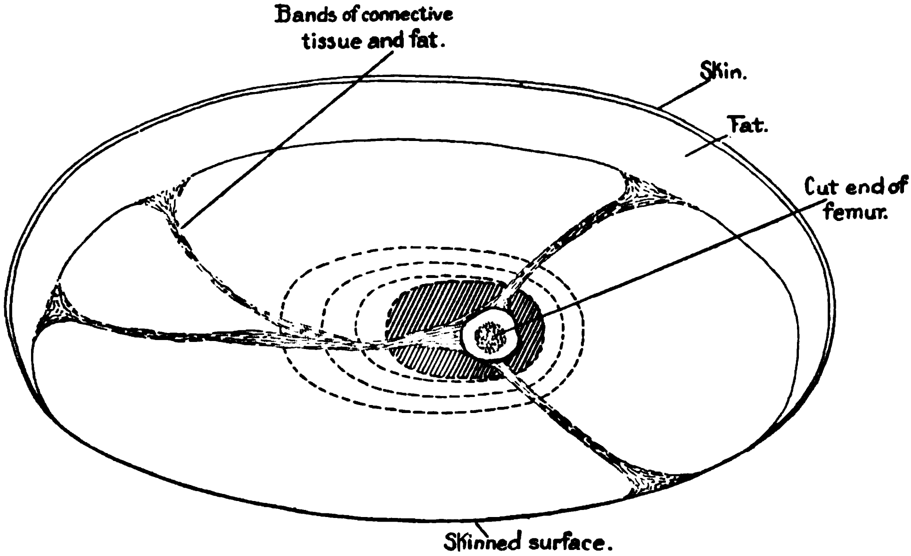

FIG. 1.—Cross section through body of ham, with sour areas indicated by shading and dotted lines.

The distribution of the sour area in the body of a well-developed sour is shown in figure 1.

In the case of a well-developed body sour the sour area is more pronounced near the bone, as represented in figure 1 by the shaded area, and may extend out into the body of the ham for a variable distance, according to the degree of souring, as represented by the dotted lines, gradually fading off toward the margins, where it may be imperceptible or entirely wanting.

In the pronounced sours, termed "stinkers," the odor pervades the entire ham, and is of a distinctly putrefactive quality.

In shank sours, the souring is more or less confined to the shank, or the region about the tibio-femoral articulation, but may extend upward into the lower portion of the body of the ham.

METHOD OF DETECTING SOUR HAMS.

Souring is detected and located by means of a pointed metal instrument known as a "ham trier," which resembles a long, slightly flattened ice pick. The trier is thrust into the ham at different points along the bone, rapidly withdrawn, and the odor which clings to the metal noted. The trained inspector works very rapidly, and is able to detect even the slightest sour or off odor which might be imperceptible to one not trained to the work. At the end of the cure all hams are tested with the trier under the supervision of Government meat inspectors.

Hams are also given what is called the "30-day inspection" by plant inspectors during the process of curing. An average ham weighing from 14 to 16 pounds requires about 60 days to cure, and at the end of 30 days a certain number of hams in each run are usually tested to see how the cure is progressing. If no sour hams are discovered at this inspection the packer knows that the cure is progressing satisfactorily, and moreover he feels sure that his hams will finish satisfactorily, for experience has taught him that souring develops within the first four weeks of the curing period, and if his hams are sweet at the end of this time, he can feel practically sure that no sours will develop later on.

THEORIES IN REGARD TO HAM SOURING.

The theories as to the cause of souring are many and varied. The majority of them are pure speculation and have no foundation upon observed facts. A few of these theories may be enumerated to show how wide and varied has been the speculation upon this subject.

A theory which is quite prevalent among packing-house employees attributes souring to overheating of the animal previous to slaughter, but tests were made by driving hogs to the point of exhaustion just prior to slaughter and curing the hams from these animals in comparison with hams taken from animals which had been rested prior to slaughter, with no difference in the cured product; that is, the hams taken from overheated hogs cured equally as well as those taken from rested hogs.

Another theory attributes souring to a diseased condition of the meat. Prior to the enforcement of the Federal regulations governing meat inspection there might have been some ground for such a supposition, but this theory could not hold at the present time, in view of the thorough and efficient inspection now in force, for it can be safely said that no diseased meat now passes the Government inspectors, and therefore no diseased meat goes into cure in inspected houses. In order to test this theory, however, hams were secured from a number of condemned animals which showed various diseased conditions, such as hog cholera, pyemia, septicemia, scirrhous chord, etc., and these hams were cured in comparison with hams taken from normal hogs. It was found that the hams taken from the diseased hogs cured equally as well as those taken from healthy hogs. The hams from the diseased hogs were destroyed after the experiment, as the meat taken from diseased animals was of course not considered fit for consumption, the object of the experiment being merely to determine whether or not souring is caused by diseased conditions.

Another theory attributes souring to imperfect or too rapid chilling of the meat before it is put in pickle, and places the blame upon the refrigeration. According to this theory, souring results when the meat is chilled too suddenly, the idea being that by the rapid congealing of the juices of the meat a coating is formed on the outside of the ham whereby the animal heat is prevented from escaping from the interior, leaving the meat next to the bone at a higher temperature than the outside of the ham.

In order to test this last theory, a number of hog carcasses were run direct from the killing floor to a cooler at 28° F. and a like number of carcasses of the same average weight which had been allowed to stand for two hours at the outside temperature of the air (53° F.) were placed in the same cooler. The carcasses which had hung for two hours in the air had lost an average of 14 degrees in temperature before going to the cooler. The temperature of the cooler rose to 29° F. after the carcasses were put in, but was soon reduced to 28° F. and held at this temperature. The temperatures of the hams were taken at the end of 24 hours, and practically no difference was found in the inside temperatures of the two lots; that is, the hams on the hot carcasses which were subjected to a sudden chilling exhibited practically the same inside temperature (i. e., next to the bone) as those which had cooled for two hours at the temperature of the air before being placed in the cooler.

Still another theory attributes souring to lack of penetration of the pickling fluids, but analyses of sour and sound hams do not seem to bear out this theory. The rate of penetration of the pickling fluids, however, would seem to have some bearing on the subject, and this point will be discussed later in connection with some laboratory experiments on the inhibitory effects of sodium chlorid and potassium nitrate.

So much for the more commonly accepted theories which have been advanced to explain ham souring.

PREVIOUS EXPERIMENTAL WORK TO DETERMINE CAUSE OF HAM SOURING.

A review of the literature reveals but one article bearing directly on the subject of the cause of ham souring.

In June, 1908, Klein[1] published in the London Lancet an article on "miscured" hams. He describes a miscured ham as one which has a distinctly putrid smell, and the tainted areas he describes as varying in color from a dirty gray to a dirty green, the muscular tissues in the strongly tainted areas being swollen and soft, or jelly-like. From such hams he isolated a large nonmotile, nonspore-bearing, anaerobic bacillus which he calls *Bacillus fœdans*. He cultivated the organism on different media and obtained from the cultures a putrid odor resembling that of the ham from which the culture was obtained, but did not attempt to produce tainting by injecting sound hams with the bacillus.

---

[1] Klein, E. On the nature and causes of taint in miscured hams. The Lancet, vol. 174, London, June 27, 1908.

While there can be little doubt that Klein's bacillus was the cause of the tainting in those hams which he examined, the proof would certainly have been stronger had he injected sound hams with cultures and thus proven that he could reproduce tainting experimentally by means of his bacillus. Klein examined only dry-cured hams and does not state the temperature at which they were cured. He fails to offer any explanation as to how the bacillus gained entrance into the hams.

THE PRESENT EXPERIMENTS.

MEDIA EMPLOYED.

After considerable experimentation as to a suitable culture medium for the bacteriological study of sour hams, a modification of the "egg-meat mixture" used by Rettger[1] in his studies on putrefaction was found to be the most satisfactory. This medium, which consists of chopped meat and egg albumen, furnishes an excellent medium for the growth of putrefactive organisms which rapidly break down the proteids of the meat, giving rise to the characteristic odors of putrid decomposition. Rettger used chopped beef and egg albumen, but for the present work chopped pork was substituted for the beef, as affording a more suitable medium for the growth of organisms accustomed to growth in pork hams. The modified medium is prepared as follows:

A. One-half pound of lean pork, freed from excess of fat and sinew, is finely chopped in a meat chopper, 250 cubic centimeters of water is then added, the meat acids are neutralized with sodium carbonate, and the mixture is heated in an Arnold sterilizer for 30 minutes, with occasional stirring. It is then set away in a cold place for several hours. A small amount of fat collects at the top in the form of a fatty scum, as it is impossible to remove all of the fat from the meat before it is chopped. The fatty scum, which hardens upon standing in the cold, is now removed.

B. The whites of three eggs are mixed with 250 cubic centimeters of water. The mixture is rendered neutral to phenolphthalein by means of dilute hydrochloric acid and heated for 30 minutes in the Arnold sterilizer, with occasional stirring.

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[1] Rettger, L. F. Studies on putrefaction. Journal of Biological Chemistry, vol. 2, 1906.

A and B are now mixed and 2.5 grams (0.5 per cent) of powdered calcium carbonate added. The mixture is next run into large sterile test tubes, or sterile flasks, and sterilized in an Arnold sterilizer on three successive days.

In addition to the egg-pork mixture described above, culture tubes of agar and bouillon prepared from pork instead of beef, with the addition of 1 per cent of glucose, were also used; but the best results were obtained with the egg-pork medium, as with this medium, the early development of sour or putrefactive odors furnished a valuable indication as to the presence of organisms capable of producing sour or putrefactive changes in meat.

METHOD OF PROCEDURE IN EXAMINING HAMS.

The hams were sectioned through the body, the femur, or "middle bone," as it is known in packing-house parlance, being cut at a point about 1½ or 2 inches below its head. A cross section of a ham thus cut is shown in figure 1. After sectioning, the hams were subjected to a microscopical, bacteriological, and chemical examination as follows:

Microscopical examination.—Bits of muscular tissue, taken from various points, were teased out in salt solution and the condition of the muscle fibers noted. Smear preparations were also made from bits of muscular tissue and from the bone marrow, and these were stained and subjected to microscopical examination. Portions of the meat were also hardened and cut into microscopic sections, which were stained and mounted for histological and bacteriological study.

Bacteriological examination.—In the bacteriological examination of sour hams, especial attention was directed to the detection of anaerobic species, as it seemed reasonable to suppose that if the changes taking place in sour hams were due to bacteria these bacteria would in all likelihood be anaerobes (i. e., organisms which develop in the absence of oxygen). This assumption was based upon the fact that, as a rule, souring begins in the interior of the ham next to the bone, and, furthermore, the hams are cured in large vats where they are completely submerged in the pickling fluids, so that any bacteria which develop within the bodies of the hams while they are in cure are probably restricted to practically anaerobic conditions.

Cultures were made from the interiors of the hams at various points by first searing the cut surface thoroughly with a heavy metal spatula and then cutting out, by means of sterile scissors and forceps, plugs of meat about 1 cm. square. The plugs of meat were then dropped into tubes containing the egg-pork medium and pushed down to the bottom of the tubes, where they were held in place by the chopped meat above; in this way conditions favorable for the development of anaerobic organisms were obtained. In inoculating the pork-agar tubes, the medium was first boiled to expel any inclosed air and cooled to 43° to 45° C.; the plugs of meat were then dropped into the tubes and the agar rapidly solidified by plunging the tubes in cold water; in this way the bits of meat were inclosed in the agar at the bottom of the tubes, affording suitable conditions for anaerobic growth. Aerobic and anaerobic plates were also made from the meat, and in most cases bouillon tubes were also inoculated. Cultures were always taken from the bone marrow as well as from the meat. Novy jars were also used for obtaining anaerobic conditions in growing the cultures.

Chemical examination.—In order to determine whether the souring was connected with or dependent upon a lack of penetration of the pickling fluids to the interior of the meat, the hams were further subjected to a chemical examination and the content of the meat in sodium chlorid and potassium nitrate determined at varying depths.

RESULTS OF EXAMINATION OF SOUR AND SOUND HAMS.

The sour hams examined were obtained from four different packing establishments. All of the hams studied were "sweet-pickle hams" which had not been smoked. The sour hams selected for examination were good typical body sours, in which the sour odor was well developed, but not of the very pronounced or putrefactive type.

The sour odor in every case was found to be more pronounced next to the bone, being usually rather more pronounced just behind the bone, that is, on the fat side of the bone. The sour odor in each instance was confined to an area of meat immediately surrounding the femur and extending out through the body of the ham for a variable distance, as shown by the dotted lines in figure 1, but in no case did the sour odor extend all the way to the margin of the meat, nor did it as a rule extend below the tibio-femoral articulation, the shank proper and the bone marrow of the shank (i. e., of the tibia) being usually sweet. The butt portion of the hams—that portion above and behind the hitch bone (symphasis pubis)—was also sweet.

Immediately after sectioning, the sour areas, as a rule, could be readily distinguished by a difference in color. In the freshly cut hams the muscular tissue near the bone, where the sour odor was more pronounced, exhibited a slight but distinct grayish hue, at times having a slight greenish tinge; in other words, the muscular tissue in the sour areas lacked the normal bright red color of the sound meat and was distinctly lighter in color than the surrounding tissues. Upon exposure to air, however, the lighter, grayish, sour areas tend to assume a reddish hue and become much less pronounced than in the freshly cut ham. After the cut surface of the ham has been exposed to the air for some time it may be difficult to distinguish the sour areas by any difference in color.

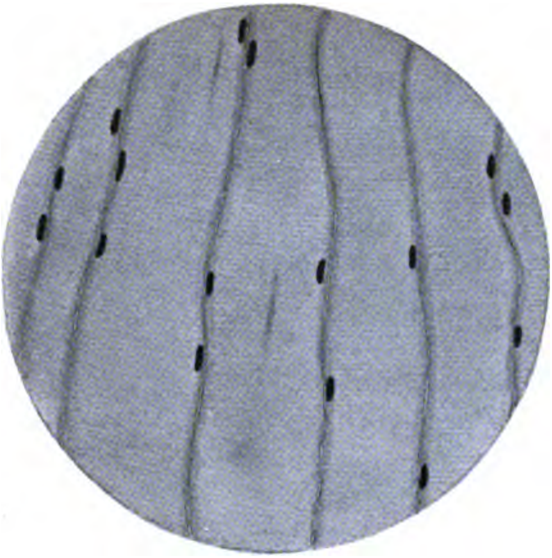

FIG. 1.—SECTION OF MUSCULAR TISSUE FROM SOUND HAM, SHOWING MUSCLE FIBERS CUT LONGITUDINALLY; NUCLEI SHARPLY DEFINED AND CROSS STRIATION DISTINCT.

(Pen-and-ink drawing made with camera lucida from section stained with hematoxylin and eosin to show histological structure. × 320.)

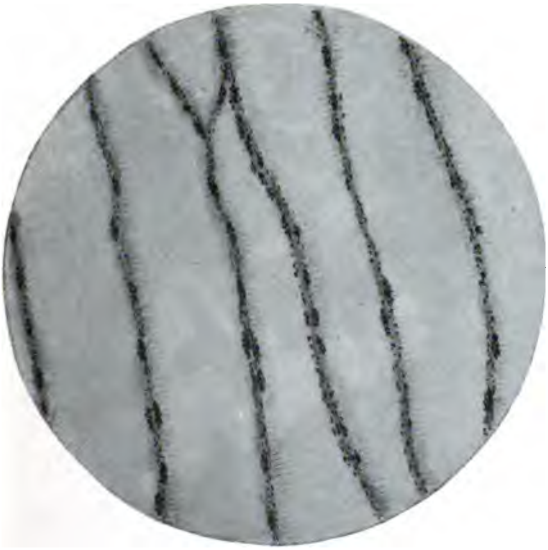

FIG. 2.—SECTION OF MUSCULAR TISSUE FROM SOUR HAM, SHOWING MUSCLE FIBERS CUT LONGITUDINALLY; NUCLEI UNDERGOING DISINTEGRATION AND CROSS STRIATION INDISTINCT.

(Pen-and-ink drawing made with camera lucida from section stained with hematoxylin and eosin to show histological structure. × 320.)

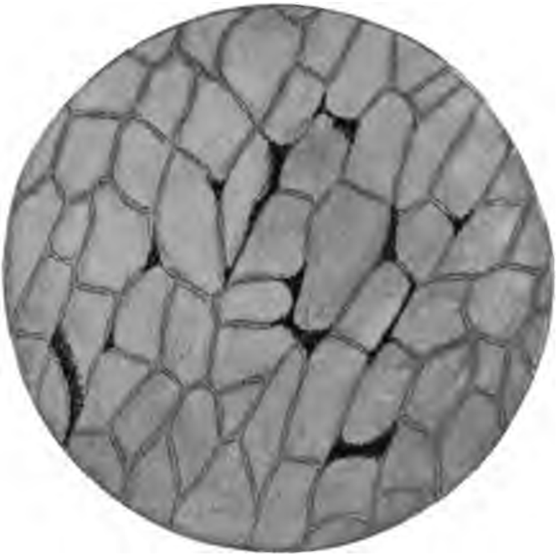

FIG. 1.—SECTION THROUGH MUSCULAR TISSUE OF HAM WHICH HAS UNDERGONE NATURAL OR SPONTANEOUS SOURING, SHOWING DISTRIBUTION OF BACILLI BETWEEN THE MUSCLE FIBERS, WHICH ARE CUT OBLIQUELY. THE DARK MASSES BETWEEN THE MUSCLE FIBERS REPRESENT CLUMPS OF BACILLI.

(Pen-and-ink drawing made with camera lucida from section stained by the Gram-Weigert method to show bacteria. × 85.)

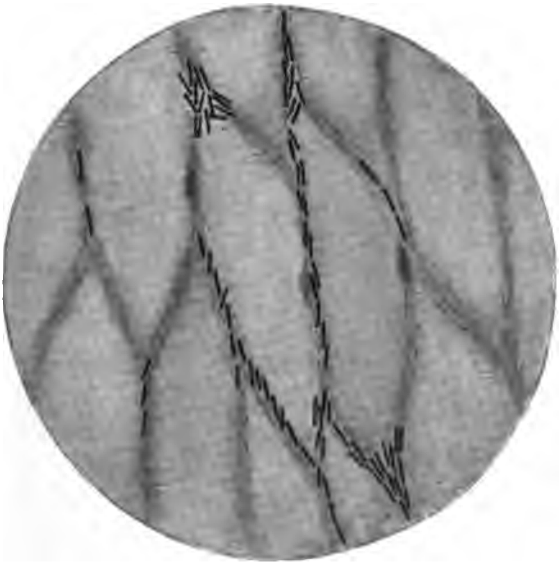

FIG. 2.—SECTION THROUGH MUSCULAR TISSUE OF HAM WHICH HAS UNDERGONE NATURAL OR SPONTANEOUS SOURING, SHOWING INDIVIDUAL BACILLI BETWEEN THE MUSCLE FIBERS, WHICH ARE CUT SOMEWHAT OBLIQUELY. NUCLEI HAVE LOST SHARP OUTLINE AND CROSS STRIATION IS INDISTINCT.

(Pen-and-ink drawing made with camera lucida from section stained by the Gram-Weigert method to show bacteria. × 320.)

In the sour areas near the bone the muscular tissue was distinctly softer; that is, it broke and cut more readily than the surrounding tissues. This was usually quite noticeable in cutting out plugs of the meat for making cultures. In a ham which shows pronounced souring the muscular tissues in the worst affected areas may become quite soft and even slightly gelatinous.

The sour areas, when tested with litmus paper, frequently showed a slight but distinct alkaline reaction. When aqueous extracts of the sour meat, however, were titrated with phenolphthalein they were found to be acid.

HISTOLOGICAL CHANGES IN SOUR HAMS.

In preparations made by teasing out bits of the meat in physiological salt solution, the cross striation of the muscle fibers from the sour areas was found to be much less distinct than in similar preparations taken from sound portions of the meat or from sound hams. At times it was found that the muscle fibers in the sour areas had completely lost their cross striæ, but the longitudinal striation could still be made out. In cases where the souring was pronounced there was sometimes complete loss of both longitudinal and cross striation; in these cases the muscle fibers appeared to have undergone slight swelling and the protoplasm exhibited a finely granular appearance.

In stained sections of the sour meat another striking change was noticed in the disintegration of the nuclei of the muscle fibers, which are at times completely broken up, appearing as bluish granular masses in sections stained with hemotoxylin and eosin. (Compare figs. 1 and 2 of Pl. I.)

In sections stained by the Gram-Weigert method to show the presence of bacteria, a large Gram-staining bacillus was noted between the muscle fibers in the connective-tissue elements of the muscle. In some of the sections these bacilli were present in great numbers, sometimes in densely packed clumps or masses, while in other sections, or in other portions of the same section, they were only sparsely distributed between the muscle fibers. Where the bacteria were more numerous the histological changes in the muscle fibers, especially the breaking down of the nuclei, were more noticeable. The intermuscular connective tissue had apparently furnished paths of least resistance along which the organism followed. In Plate II, figures 1 and 2, the bacteria are shown between the muscle fibers under low and high power magnifications.

In Plate II, figure 1, under the low-power magnification, the bacteria appear as dark clumps or bands between the muscle bundles. Under the high power they are shown following along the sarcolemma sheaths between the muscle fibers.

CHEMICAL ANALYSES OF SOUR AND SOUND HAMS.

In order to determine whether there was any difference in regard to the penetration of the pickling fluids in the sour hams as compared with sound hams, a series of four sour hams were subjected to a chemical examination in comparison with four sound hams. All were sweet-pickle hams and were obtained from the same packing establishment. They were all of the same cure and the same approximate age (i. e., length of cure) and the same approximate weight.

In taking samples for chemical analysis, the following procedure was adopted: A section about 2½ inches wide was cut from the center of the body. The two ends of this section were then trimmed off along the lines L-M and N-O, as shown in figure 2. Beginning at the skinned surface, four slices, A, B, C, and D, were then made, as indicated by the dotted lines. Slice B contained the bone in each instance. Slice D was practically all fat. Each slice was ground separately in a meat chopper and the sample thoroughly mixed before taking out portions for analysis.

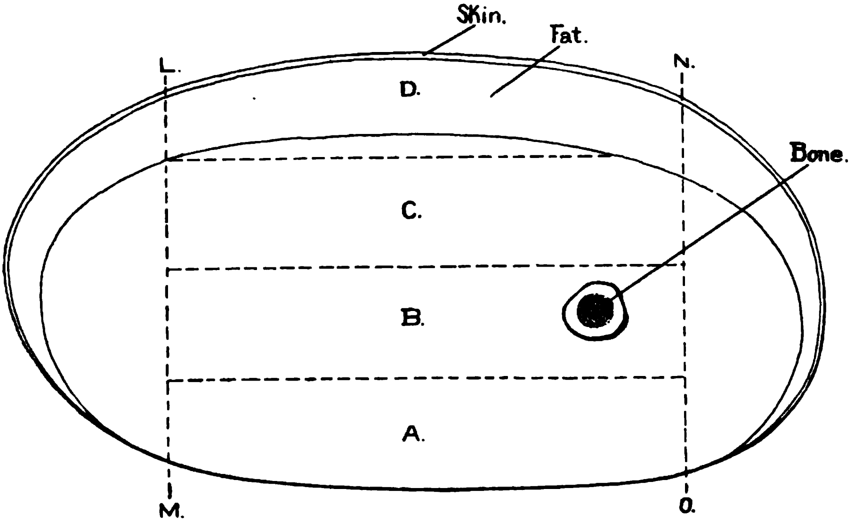

FIG. 2.—Cross section through body of ham to show method of sampling for chemical analysis. A, slice below bone; B, bone slice; C, slice above bone; D, fat slice.

As all of the hams examined were mild-cure hams, that is, had been pumped in the shank only, the pickling fluids in order to reach the bodies of these hams had to penetrate chiefly from the skinned surface of the ham, as little if any penetration takes place through the thick skin of the ham.

The analyses[1] shown in the following tables therefore indicate the degree of penetration of the pickling fluids.

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[1] These analyses were made by Mr. R. R. Henley, of the Biochemic Division, Bureau of Animal Industry

Analyses of sour hams.

No.	Description.	Slice.	NaCl.	KNO_3.
			Per cent.	*Per cent.*
1	Sour body	A	6.18	0.175
		B	4.83	.224
		C	3.65	.299
		D	1.03	.074
2	....do	A	5.34	.174
		B	3.70	.150
		C	2.79	.174
		D	1.12	.012
3	....do	A	5.04	.125
		B	4.08	.149
		C	2.72	.099
		D	1.19	.048
4	....do	A	7.78	.250
		B	5.31	.100
		C	4.76	.200
		D	1.96	.048

Analyses of sound hams.

No.	Description.	Slice.	NaCl.	KNO_3.
			Per cent.	*Per cent.*
1	Sound	A	5.80	0.211
		B	4.83	.188
		C	3.86	.221
		D	1.33	.063
2	....do	A	4.94	.197
		B	4.08	.149
		C	3.05	.223
		D	1.56	.059
3	....do	A	5.92	.173
		B	4.29	.099
		C	4.12	.139
		D	2.32	.049
4	....do	A	5.53	.119
		B	4.89	.079
		C	4.32	.099
		D	2.19	.041

Taking an average of the four slices in each ham so as to get an average for the entire ham, and comparing the sour hams with the sound hams, we have the following comparison:

	NaCl.	KNO_3.
Average for 4 sour hams (entire ham).......per cent..	3.84	0.143
Average for 4 sound hams (entire ham).........do....	3.93	.131

These figures show practically no difference between the sour and the sound hams as regards the sodium chlorid and potassium nitrate content of the entire ham.

If, now, we compare the bone slices—and these afford really a better basis for comparison, as in sour-body hams the souring is always more pronounced around the bone—we have the following figures:

	NaCl.	KNO_3.
Average for 4 sour hams (bone slice)........per cent..	4.48	0.155
Average for 4 sound hams (bone slice)..........do....	4.52	.129

Here, again, we find no essential difference between the sour and the sound hams, and we must conclude from these analyses that souring does not depend upon or result from a lack of penetration of the pickling fluids.

It seems probable that in mild-cure hams, which are pumped in the shank only, the souring begins in the upper portion of the shank and extends upward along the bone into the body of the ham, and that it takes place before the pickling fluid has penetrated to the interior of the ham. When the pickling fluid reaches the interior of the ham it tends to inhibit the souring, which, as will be shown later, is due to the development of bacteria within the bodies of the hams. The growth of the bacteria, however, within the bodies of the hams and the histological changes in the muscle fibers do not seem to interfere with the penetration of the pickling fluids.

BACTERIOLOGICAL EXAMINATION OF SOUR AND SOUND HAMS.

In all of the sour hams which were examined bacteriologically a large anaerobic bacillus was found to be constantly present. From several of the hams this bacillus was obtained in pure culture; that is, it was the only organism present in cultures made from the sour meat and from the bone marrow of the femur. Such cultures, when held at room temperature, gave, at three days, a sour-meat odor exactly resembling that obtained from sour hams.

In many of the sour hams other bacteria were found in association with the anaerobic bacillus noted above. These other bacteria, however, were not constant, being sometimes present and sometimes absent. Among the other bacteria noted in the sour hams, the following forms occurred most frequently:

1. A nonmotile, gram-positive bacillus, measuring from 1.5 to 4 microns in length by 0.5 micron in breadth, sometimes in chains and filaments.
2. A small, nonmotile, gram-negative bacillus, about the size of *Bacillus coli* and usually in pairs.
3. A large micrococcus.

Sometimes one and sometimes all of these bacteria were present in a given ham. They were encountered most frequently in hams which had been pumped in both body and shank, and were probably ordinary pickle bacteria. They were not strict anaerobes, but belonged to the class of facultative or optional anaerobes; that is, organisms which will grow either with or without free oxygen. These bacteria were isolated and grown on the egg-pork medium, but failed to give any characteristic sour or putrefactive odors, and were therefore discarded.

A series of sound hams, all of them of mild cure—that is, hams which had been pumped in the shank only—were also examined bacteriologically. In examining these hams cultures were taken at varying depths, beginning at the skinned surface and going backward toward the fat. Cultures were also taken from the bone marrow of the femur. In the cultures taken near the skinned surfaces the ordinary pickle bacteria were obtained, but these did not, as a rule, extend beyond a depth of 3 centimeters below the skinned surface. The cultures taken from the deeper portions of the hams and from the bone marrow of the femur were entirely negative—that is, failed to show any growth—and the anaerobic bacillus noted in the sour hams was not encountered in any of the cultures made from these hams.

The anaerobic bacillus isolated from the sour hams was found to correspond in morphology with the organism noted in the microscopic sections made from the muscular tissue. In view of this fact and the fact that it was constantly present in the sour hams examined, and was capable of producing in egg-pork cultures a sour-meat odor of the same nature as that obtained from sour hams, this organism was subjected to further study and experimentation.

INOCULATION EXPERIMENTS WITH HAMS.

The experiments which follow were conducted at two different packing establishments in one of the larger packing centers of the country. The officials at each of these establishments showed great interest in the experiments and were most courteous and obliging in supplying the necessary materials.

The first question to be decided was whether the bacillus isolated from sour hams was actually capable of causing ham souring. The bacillus in question had, when cultivated on the egg-pork medium, given rise to a sour odor similar to that obtained from sour hams, but this was not regarded as proof positive that the organism was the actual cause of souring in hams. The proper way to decide this point seemed to be to inoculate hams with the bacillus and then subject these hams to the regular method of cure and see whether they became sour, just as the pathogenic properties of a disease-producing organism are determined by the inoculation of experiment animals. The first two experiments which follow were designed to decide this point.

It was regarded as important to conduct similar experiments at two different establishments, in order to determine whether the same results would be obtained under the somewhat different conditions imposed by different methods of cure. The two experiments which follow were carried out, therefore, at different establishments.

Experiment I.

In carrying out this experiment four tierces of hams were "put down" or "packed"—that is, placed in cure. Two of the tierces were given the fancy or mild cure and two the regular or stronger cure. The hams in two of the tierces, one mild and one regular cure, were injected with a culture suspension of the bacillus; the other two tierces were not injected with culture and were put down to serve as checks on the cure. Hams weighing from 12 to 14 pounds were used for the mild cure, while for the regular cure hams weighing from 14 to 16 pounds were used. This was in accordance with the general rule which prevails in packing houses, the lighter hams being subjected to the mild cure and the heavier hams to the regular cure. The only difference between the mild and the regular cure in this experiment lay in the pumping. The hams which were given the mild cure were pumped in the shank only, while those given the regular cure were pumped in the body as well as in the shank.

All of the hams had received the usual 48-hour chill. They were all pumped with the same pumping pickle and cured in the same curing pickle, and were in cure for the same length of time. The pumping and curing pickles used were the regular pumping and curing pickles of the establishment at which the experiment was carried out, and the hams were cured in accordance with the fancy and regular cures as practiced at this establishment.

The hams were packed in new tierces which had been thoroughly scalded with boiling water. The tierces were held in a curing room which was kept at an average temperature of from 34° to 36° F., the temperature occasionally going as high as 38° and 40° F., but never above 40° F. The hams were left in cure for about 70 days, which is a little longer than the usual cure. The tierces were rolled three times during the cure. At the end of the cure the hams in all four tierces were carefully tested by an expert meat inspector, who knew nothing of the treatment which the hams had received.

The hams in two of the tierces were inoculated with a culture suspension prepared as follows: Ten tubes of egg-pork medium, each tube containing approximately 10 cubic centimeters of the medium, were inoculated with the bacillus and held at room temperature (20° to 25° C.) for six days. The cultures were then filtered through sterile gauze into a large sterile flask; this was done in order to remove the particles of meat, which might otherwise have clogged the syringes used in inoculating the hams. In transferring the contents of the culture tubes to the filter the tubes were washed out with sterile physiological salt solution (0.6 per cent sodium chlorid), and the meat particles on the filter were afterwards washed with the salt solution, a sufficient quantity of the latter being used to bring the total volume of filtrate to 400 cubic centimeters. A microscopic preparation from the filtrate showed the organisms in large numbers, with an occasional rod showing a large terminal spore. This suspension was used for the injection of 40 hams, each ham being given 10 cubic centimeters, or the equivalent of 2.5 cubic centimeters of the original culture. The hams were injected with the culture suspension by means of a sterile syringe carrying a long 5-inch needle. The needle was thrust well into the body of the ham at a point near the upper end of the middle bone or femur, the latter being used as a guide in inserting the needle and the injection being made into the tissues just behind and a little to one side of the upper end of the femur.

The details of the experiment were as follows:

Tierce No. 1 (fancy cure).—This tierce contained 20 hams weighing from 12 to 14 pounds each. These hams were pumped in the shank only. Immediately after pumping they were injected with 10 cubic centimeters each of the liquid culture or suspension described above. After injection the hams were immediately packed in the tierce, which was then headed up, filled with the regular curing pickle, and placed in cure.

Result: When tested at the end of the cure all of the hams in this tierce save one were found to be sour. In 10 of them the souring was very marked throughout the body of the ham and extended into the shank as well. In six the souring was very marked in the body of the ham, but did not extend into the shank. In three there was slight but well-marked souring in the body of the ham with no souring in the shank, and one remained sweet. The probable explanation of the variation in the degree and the extent of the souring will be discussed later. The bone marrow of the femur or middle bone was tested in all of the hams and found to be sour in 18. In one of the hams which showed only slight souring in the body the souring did not extend through to the bone marrow, and in the ham which remained sweet the bone marrow was also sweet. The fact that one ham in this tierce remained sweet was in all likelihood due to an oversight in making the inoculations. In making the inoculations the hams were spread out in a row on a table by a packing-house assistant, who removed the hams as soon as they were inoculated and placed them in tierces; and it is more than probable that the assistant removed one of the hams before it was inoculated in the interval when the writer was busy filling the syringe for the next inoculation.

Tierce No. 2 (fancy cure).—This tierce contained 20 hams of the same average weight as the preceding. They were pumped in the shank only, but were not injected with culture, being put down to serve as checks on the hams in tierce No. 1. These hams, therefore, were subjected to exactly the same cure and were held under exactly the same conditions as those in tierce No. 1, the only difference being that the hams in this tierce were not injected with culture.

Result: When tested at the end of the cure all of the hams in this tierce were found to be perfectly sound and sweet, showing that the curing in this instance was properly carried out and that the souring of the hams in tierce No. 1 was undoubtedly due to the injections of culture which they received.

Tierce No. 3 (regular cure).—This tierce contained 20 hams weighing from 14 to 16 pounds each. These hams were pumped in the shank and also in the body. Immediately after pumping they were each injected in the same manner as those in tierce No. 1 with 10 cubic centimeters of culture. The hams were then packed in tierce and placed in cure.

Result: At the end of the cure 9 of the hams were found to be sour, while 11 remained sweet. Of the 9 hams which became sour, 1 showed very pronounced souring in the body and in the shank as well, 3 showed very pronounced souring in the body, 1 showed pronounced souring in the body, and 4 slight souring in the body. The bone marrow of the femur was tested in all of the sour hams and was found to be sour in 7. In 2 of the sour hams which showed slight souring in the body the souring noted in the meat had not extended through to the bone marrow.

Tierce No. 4 (regular cure).—This tierce contained 20 hams of the same average weight as those in tierce No. 3, and, like the latter, were pumped in both shank and body, but were not injected with culture. This tierce was put down to serve as a check on tierce No. 3 and was held under exactly the same conditions, the only difference being that these hams were not injected with culture.

Result: At the end of the cure the hams were carefully tested and all were found to be perfectly sound and sweet.

Results of Experiment I.

No. of tierce.	Number of hams.	Average weight of hams.	Cure.	How pumped.	Treatment.	Condition at end of cure.	
						Number of sour hams.	Percentage of sour hams.
		Pounds.					
1	20	12-14	Fancy..	Shank only.......	Each ham injected with 10 c. c. of culture.	19	95
2	20	12-14	...do.....	.....do............	Not injected with culture; check on tierce 1.	0	0
3	20	14-16	Regular..	Shank and body..	Each ham injected with 10 c. c. of culture.	9	45
4	20	14-16	...do.....	.....do............	Not injected with culture; check on tierce 3.	0	0

Three hams from each tierce were selected for bacteriological and histological examination. From tierces 1 and 3, which contained the injected hams, three of the most pronounced "sours" were selected from each tierce. In examining the hams bacteriologically the following method was adopted: The hams were sectioned near the center of the body and the larger or butt end turned up so as to expose the cut surface. A cross section of a ham thus cut is shown in figure 3.

Cultures were taken at the points indicated by the numbers and from the exposed bone marrow of the femur by first searing the surface, and then taking out plugs of the meat or marrow by means of sterile instruments. The plugs of meat or marrow were dropped into tubes containing egg-pork medium and pushed to the bottom of the tubes by means of a sterile platinum wire. In the cultures made from the sour hams from tierces 1 and 3, which were injected with culture, the bacillus with which these hams were injected was found in practically every culture, although it was sometimes absent in the cultures taken at points near the skinned surfaces of the hams (i. e., at points 1, 4, and 5 in fig. 3). In the cultures taken from the meat, the bacillus was not always present in pure culture, but this is not to be wondered at when we remember that the pickling fluids often contain large numbers of bacteria of various kinds, and these, of course, find their way into the hams in the pickling fluids. Especially is this true of the hams which are pumped in the body, where bacteria are actually pumped into the bodies of the hams in the pumping pickle. In the case of hams which are not pumped in the body, the pickle bacteria do not appear to penetrate the body of the ham to any great depth.

In figure 3 the plus signs after the figures represent the distribution of the sour-ham bacillus in one of the hams from tierce 1, and this may be taken as a typical example of the other sour hams which were examined in this experiment. It should be explained that the shaded areas are not intended to represent the actual limits of souring, but simply the areas in which the sour odor was most pronounced and from which it could be readily obtained with the trier. In comparing the regular and mild cure hams, it was found that the areas of souring as defined with the trier were more restricted in the regular cure hams, and this was undoubtedly due to the additional pumping which these hams received, whereby the growth of the bacillus was partially inhibited.

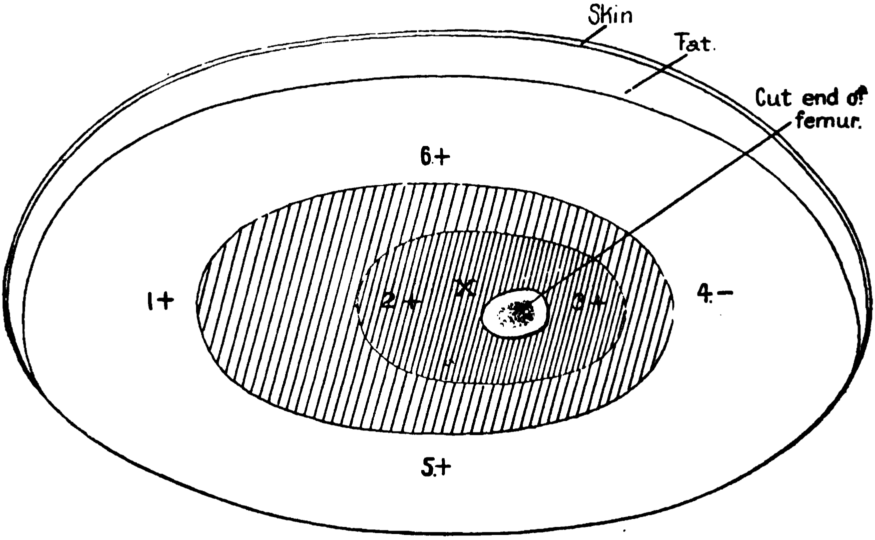


FIG. 3.—Cross section through body of artificially soured ham, showing sour areas and points at which cultures were taken. Darker shading indicates sour area in hams pumped in body and shank; light shading indicates sour area in hams pumped in shank only; figures indicate points at which cultures were taken; plus signs indicate presence of bacillus; minus sign indicates absence of bacillus; X indicates point of inoculation.

It will be noticed that the sour-ham bacillus was present in cultures taken at points outside the shaded areas, indicating that the organism had extended generally throughout the bodies of the hams. As the hams were inoculated at a point just to one side of and a little behind the femur (i. e., at the point X in the figure), the presence of the bacillus generally throughout the hams would indicate a very extensive multiplication of the original bacilli with which the hams were injected. In view of the fact that the bacillus in question is nonmotile, the spread of the bacilli throughout the hams must result simply from subdivision and growth by extension, and in spreading throughout the hams the bacilli appear to follow along the connective tissue bands which afford paths of least resistance. In the cultures made from the bone marrow the bacillus was recovered in pure culture from each of the hams examined, and it is probable that the bacillus finds its way into the bone marrow from the meat by following along the small arteries which pass through the bone. The fact that the bacillus was found in pure culture (i. e., uncontaminated) in the cultures made from the bone marrow is explained probably by its capacity for growth by extension, and also by the fact that the pickling solutions probably do not reach the bone marrow until late in the curing and then only to a limited extent. The bacteria which ordinarily occur in pickling fluids are not strict anaerobes and are not placed under the most suitable conditions for growth when they reach the interior of the ham, for it seems probable that in the interior of hams which are totally submerged in pickling fluids the amount of available oxygen must be extremely small. The ordinary pickle bacteria, therefore, would not multiply as rapidly in the interior of the hams and would not find their way into the bone marrow as soon as would a strictly anaerobic organism.

Pure cultures of the sour-ham bacillus, recovered from the meat and bone marrow of the injected hams, were compared with cultures of the original bacillus used for inoculating the hams, and were found to be identical. Furthermore, the bacillus with which the hams were injected was recovered from the injected hams at points far removed from the original point of injection, showing that the organism had multiplied and extended throughout the bodies of the hams and that it was clearly responsible for the souring which the hams had undergone.

Sound hams from tierces 2 and 4 were examined bacteriologically in the same manner as the injected hams, and some of the cultures showed the ordinary pickle bacteria, but in not a single instance did egg-pork cultures yield a sour odor, and in no case could the sour-ham bacillus be demonstrated in any of these hams.

Microscopic sections and teased preparations of the muscle fibers in salt solution were prepared from several of the sour hams in this experiment, and these preparations showed the same histological changes and the same distribution of bacilli as noted in the natural sours.

In Plate III, figures 1 and 2, sections are shown of artificially soured hams, that is, hams which were artificially soured by injections of culture; and if these figures be compared with the sections made from hams which had undergone spontaneous souring (see Pl. II, figs. 1 and 2) the similarity in the form and distribution of the bacilli will be at once apparent.

Summary and discussion of Experiment I.—Comparing tierces 1 and 2, where the hams were pumped in the shank only, the only difference being that the hams in tierce 1 were inoculated with culture while those in tierce 2 were not, we find that in tierce 1 nineteen out

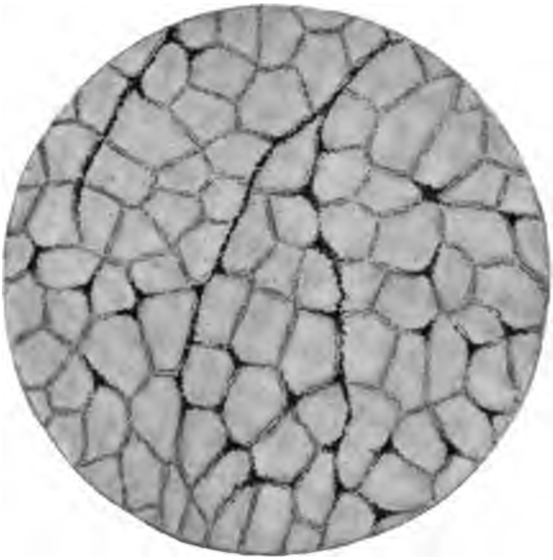


FIG. 1.—SECTION THROUGH MUSCULAR TISSUE OF ARTIFICIALLY SOURED HAM, SHOWING DISTRIBUTION OF BACILLI BETWEEN THE MUSCLE FIBERS, WHICH ARE SHOWN IN CROSS SECTION. THE DARK LINES AND MASSES BETWEEN THE MUSCLE FIBERS REPRESENT CLUMPS OF BACILLI.

(Pen-and-ink drawing made with camera lucida from section stained by the Gram-Weigert method to show bacteria. × 85.)

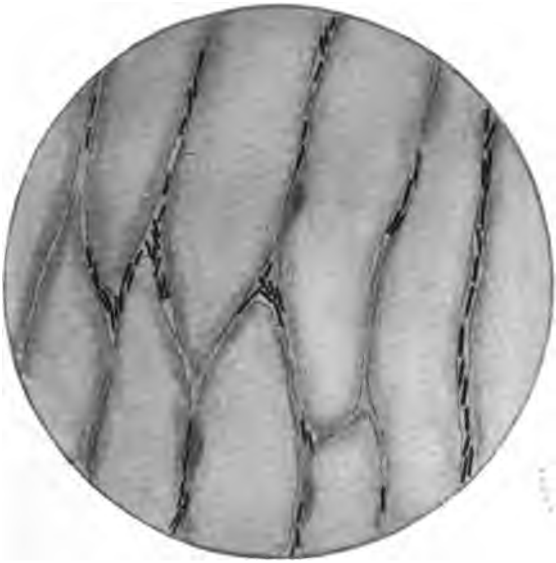


FIG. 2.—SECTION THROUGH MUSCULAR TISSUE OF ARTIFICIALLY SOURED HAM, SHOWING INDIVIDUAL BACILLI BETWEEN THE MUSCLE FIBERS, WHICH ARE CUT LONGITUDINALLY.

(Pen-and-ink drawing made with camera lucida from section stained by the Gram-Weigert method to show bacteria. × 320.)

M 40 U

of twenty, or 95 per cent, of the hams became sour, whereas in tierce 2 all of the hams remained sweet. In view of the fact that these tierces were held under exactly the same conditions, we must conclude that the souring of the hams in tierce 1 was due to the injection of culture which they received.

Comparing tierces 3 and 4, where the hams were pumped in both shank and body, the hams in tierce 3 being injected with culture while those in tierce 4 were not, we find that in tierce 3 nine out of twenty, or 45 per cent, of the hams became sour, whereas in tierce 4 all of the hams remained sweet. As the conditions of cure were the same for all four tierces, we must again conclude that the souring of the hams in tierce 3 was directly attributable to the injections of culture which they received.

If now we compare tierces 1 and 3, the two tierces which were injected with culture, we find that in the case of tierce 1, where the hams were pumped in the shank only, 95 per cent became sour, whereas in the case of tierce 3, where the hams were pumped in both shank and body, only 45 per cent became sour. In other words, the percentage of souring in those hams which were pumped in the body as well as in the shank was 50 per cent less than in those hams which were pumped in the shank only. Inasmuch as the only difference in the treatment accorded tierces 1 and 3 lay in the additional pumping given the hams in tierce 3, we must conclude that the marked diminution in the percentage of souring in the case of tierce 3 was undoubtedly due to the additional pumping which these hams received, the hams being saturated at the start with the pumping pickle. It will be shown later that both sodium chlorid and potassium nitrate exert an inhibitory effect upon the bacillus with which the hams were injected, which directly bears out the foregoing conclusion.

In tierces 2 and 4, the two check tierces which were not injected with culture, all of the hams were sweet at the end of the cure, showing that the conditions under which the experiment was carried out were entirely favorable to a successful cure.

The sour odor obtained from the artificially soured hams in this experiment was pronounced by the meat inspector who tested the hams, and who was entirely unaware of the treatment they had received, to be identical with the usual sour odor which characterizes hams that have undergone spontaneous souring; in other words, there was no difference in odor between these artificially soured hams and natural sours.

With regard to the variation in the degree and the extent of the souring exhibited by the individual hams in the two inoculated tierces, where some of the hams showed pronounced souring throughout the body and shank, while others which had been injected with the same amount of culture showed only slight souring in the body, several factors must be considered, viz: (1) Differences in the reaction of the meat of the individual hams which may have exerted an influence on the growth of the bacteria with which the hams were injected. (2) Variations in the texture of the muscle fibers and connective tissue of the individual hams, permitting in some cases a more rapid and thorough penetration of the pickling fluids to the interior of the hams, whereby the inhibitory effect of the sodium chlorid and the potassium nitrate on the bacteria would come into play earlier. (3) Variations in pumping, whereby more of the pickling solution was forced into some of the hams than into others. Probably all three of these factors would have to be taken into account in explaining the variation in the degree and extent of the souring exhibited by the injected hams.

With regard to the souring of the bone marrow, we find that of nineteen sour hams in tierce 1 eighteen showed sour marrows, while in tierce 3 nine sour hams showed seven sour marrows. The high proportion of marrow sours is not surprising when it is recalled that of the nineteen sour hams in tierce 1 the meat was markedly sour in sixteen, while of the nine sour hams in tierce 3 the meat was markedly sour in five. In the case of the four sour hams in tierce 3 which showed slight souring in the body, two of these showed sour marrows, while in two the marrows were sweet. In this experiment the percentage of sour hams showing sour marrows corresponds with the percentage of marrow-sour hams found in the packing house, where, as has been pointed out before, a ham which is markedly sour in the body will practically always show sour marrow, while in hams which show only slight souring in the body the marrow is involved in about 50 per cent of the cases.

Experiment II.

This experiment was essentially a repetition of Experiment I, but was carried out at a different packing establishment and under somewhat different conditions.

Two lots of hams were injected with a culture suspension of the bacillus at different stages of the cure, or rather at different stages in the preparation for cure, i. e., (1) on the hanging floor, previous to chilling, and (2) after chilling and pumping and immediately before packing. Three tierces, each containing 20 hams, were put down. Two of the tierces contained the hams injected with culture, while the third tierce contained check hams which had not been treated with culture. Half of the hams in each tierce were pumped in the shank, while the other half were pumped in both body and shank. The same pumping and curing pickles were used for all three tierces, and were the regular pumping and regular curing pickles of the establishment at which the experiment was carried out. The hams used were all 14 to 16 pounds in weight and were subjected to the usual 48-hour chill with an additional chill of 48 hours after they were cut from the carcass. They were packed in tierces which had been thoroughly scrubbed and cleaned with boiling water. The tierces were held in a pickling room at a temperature of 33° to 36° F., the temperature never rising above 36° F., and were rolled three times during the curing period. The hams were in cure for about eighty days. At the end of the cure the hams were carefully tested by a trained meat inspector, who knew nothing of the treatment they had received.

The culture suspension was prepared from 20 tubes of egg-pork medium in the same manner as that used in Experiment I, the cultures being diluted with sufficient salt solution to give 400 cubic centimeters of suspension. The cultures from which the suspension was prepared had grown at room temperature for ten days. The suspension was examined microscopically and showed large numbers of the bacilli in the form of filaments or long chains, with many of the individual organisms showing large terminal spores. The hams were injected with the culture suspension in the same manner as those in Experiment I.

The details of the experiment were as follows:

Tierce No. 1.—Contained 20 hams, each ham being injected with 20 cubic centimeters of the suspension or the equivalent of 10 cubic centimeters of the original culture. The hams were injected while on the hanging floor, before they had been cut from the carcasses and previous to chilling. The carcasses were still quite warm—that is, had lost but little of their body heat when the injections were made. The carcasses, which had been carefully tagged, were then run into coolers and given the usual 48-hour chill, after which the hams were severed from the carcasses and given an additional 48-hour chill in accordance with the custom of the packing house at which the experiment was carried out. The hams were next pumped with regular pumping pickle, 10 being pumped in both body and shank and 10 in shank only. They were finally packed in a tierce, which was then headed up, filled with regular curing pickle, and placed in cure.

Result: When tested at the end of the cure it was found that the 10 hams which were pumped in the shank only were all sour. In each of them the souring extended throughout the entire ham, in the shank as well as in the body, and was very pronounced, so much so that they were characterized as "stinkers" by the meat inspector who assisted in testing them. The bone marrow of the femur or middle bone was sour in all of these hams. Of the 10 hams which were pumped in both body and shank 7 showed well-marked souring throughout the body, but the souring did not extend into the shank. The bone marrow of the femur was found to be sour in 6 of these hams, while in 1 the souring had not extended through to the bone marrow.

Tierce No. 2.—Contained 20 hams which were chilled and pumped in exactly the same manner as those in tierce No. 1. These hams were injected with culture after they had been chilled and pumped, or just before they were placed in cure. The hams in this tierce, therefore, were injected with culture four days later than those in tierce 1. The hams were injected with a bacterial suspension prepared in the same manner as that used for tierce 1, except that the egg-pork cultures from which the suspension was prepared were 7 days instead of 10 days old. Each ham was injected with 20 cubic centimeters of the suspension or the equivalent of 10 cubic centimeters of the original culture. The hams were injected in the same manner as those in tierce 1.

Result: When tested at the end of the cure, it was found that of the 10 hams which were pumped in the shank all were sour; in 8 of these the souring was very marked throughout the body of the ham and extended into the shank; in all of these hams the souring had extended through to the bone marrow of the middle bone or femur. Of the 10 hams which were pumped in both body and shank 6 were sour in the body. These hams were classed by the meat inspector who examined them as "light body sours," and in none of them did the souring extend into the shank or through the bone into the bone marrow of the femur.

Tierce No. 3.—Contained 20 hams which were chilled and pumped in the same manner as those in the two preceding tierces. These hams were not injected with culture and were put down to serve as checks on the cure. In other words, they were pumped with the same pickling fluids, were subjected to exactly the same cure, and were held under precisely the same conditions as those in the preceding tierces, the only difference being that the hams in this tierce were not injected with culture.

Result: When tested at the end of the cure, all of the hams in this tierce were found to be perfectly sound and sweet.

Results of Experiment II.

No. of tierce.	Number of hams.	Average weight of hams.	How pumped.	Treatment.	Condition at end of cure.	
					Number of sour hams.	Percentage of sour hams.
		Pounds.				
1	20	14–16	10 in shank	Each ham injected with 20 c. c. of culture prior to chilling and pumping.	10	100
			10 in body and shank	do	7	70
2	20	14–16	10 in shank	Each ham injected with 20 c. c. of culture subsequent to chilling and pumping.	10	100
			10 in body and shank	do	6	60
3	20	14–16	10 in shank	Not injected with culture	0	0
			10 in body and shank	do	0	0

Four hams were selected from each tierce for bacteriological and histological examination. From tierces 1 and 2, in which the hams were injected with culture, 4 of the sourest hams were selected from each tierce. Cultures were made from these hams in the same manner as described under Experiment I and with the same result—that is, the sour-ham bacillus was found throughout the bodies of the hams. Microscopic sections were also prepared from these hams and showed the same histological changes and the same distribution of bacilli as noted for the hams in Experiment I.

Summary and discussion of Experiment II.—Comparing tierces 1 and 2, in which the hams were injected with culture, with tierce 3, where the hams were not injected with culture, we find that in tierce 1 seventeen hams (85 per cent) became sour and in tierce 2 sixteen hams (80 per cent) became sour, whereas in tierce 3 all of the hams were sweet. The fact that all of the hams in tierce 3, the check tierce, were sweet indicates that the conditions were favorable for a successful cure; and as all three tierces were cured under exactly the same conditions, the only difference being that the hams in tierces 1 and 2 were injected with culture, whereas those in tierce 3 were not injected with culture, we must conclude that the souring of the hams in tierces 1 and 2 was due to the injections of culture which they received.

Comparing tierce 1 with tierce 2, we find that the hams in tierce 1 showed more extensive souring than did those in tierce 2, this being especially noticeable in the case of the hams which were pumped in both body and shank. This difference in the extent or degree of souring was probably due to the fact that the hams in tierce 1 were injected while they were still warm and before they had lost their animal heat, the bacterial suspension thus having a better chance to become disseminated through the meat. The hams in tierce 2 were injected with culture after they had been chilled, when the tissues were more or less contracted and the conditions less favorable for the dissemination of the suspension throughout the meat. The hams in tierce 1 were also injected four days earlier than those in tierce 2, and prior to pumping; and this would explain the greater difference in the extent of the souring in the case of the hams which were pumped in both body and shank, as in tierce 1 the bacteria had four days in which to develop before coming in contact with the pickling fluids, whereas in tierce 2 the bacteria were injected after the hams were pumped with pickle and were thus brought into immediate contact with the pickling fluids, which, as will be shown later, have a distinct inhibitory action upon the bacillus in question. In the case of the hams which were pumped in the shank but not in the body there was not this difference, as in these hams the pickling fluids must penetrate into the bodies of the hams from the outside. As it requires some time for the pickling fluids to reach the interior of a ham, the bacteria were thus afforded quite an interval in which to develop before being exposed to the inhibitory action of the pickling fluids. A chemical study of the processes involved in ham curing has been carried out in the Biochemic Division and the approximate rate of penetration of the curing pickle determined, and it was found that it required about four weeks for the interior of a 10-pound ham which had not been pumped to acquire its maximum percentage of sodium chlorid.

To recapitulate: In this experiment 40 hams were injected with culture, half of this number being pumped in the shank only and half in both body and shank. Of the 20 which were pumped in the shank only, every ham without exception, or 100 per cent, became sour. Of those which were pumped in both body and shank, 13, or

65 per cent, became sour. The reduction in the percentage of sours in the last lot was clearly due to the additional pumping which these hams received.

If now we compare tierce 2 in this experiment with tierces 1 and 3 in Experiment I—these three tierces being comparable, as they were all injected with culture at the same stage in their preparation for cure, that is, subsequent to chilling and pumping—we find, in the case of the hams pumped in both body and shank, 65 per cent of sours in Experiment II as against 45 per cent in Experiment I, and this difference is undoubtedly due to the heavier dose of culture used in Experiment II, where the hams were given the equivalent of 10 cubic centimeters of egg-pork culture as against 2½ cubic centimeters in Experiment I. In the case of the hams which were pumped in the shank but not in the body, the percentage of sours was practically the same in the two experiments—in Experiment I all but one of these hams became sour, while in Experiment II all of them became sour. The degree or extent of the souring in these last hams, however, was greater in Experiment II, a result of the heavier injections of culture which they received.

Summary of Experiments I and II.

Summarizing the results obtained in Experiments I and II, we find that culture suspensions of the anaerobic bacillus isolated from sour hams caused souring with great uniformity when injected into the bodies of sound hams which were pumped in the shank only. In the two experiments, 40 sound hams which were pumped in the shank only were injected with culture suspensions of the bacillus, with the result that 39, or 97.5 per cent, became sour during the process of cure; and it is quite probable, as we have pointed out before, that one of these hams was overlooked in making the inoculations, otherwise the entire lot would have become sour.

The inhibitory action of the pickling fluids upon the bacillus is well shown in the case of those hams which were pumped in both body and shank. Out of 40 hams which were pumped in both body and shank, 22, or 55 per cent, became sour in the process of curing. Inasmuch as these hams were cured under precisely the same conditions as the hams which were pumped in the shank only, we must conclude that the diminution in souring in these hams was undoubtedly due to the additional pumping which they received, whereby the bacteria with which these hams were injected were brought into immediate contact with the strong pumping pickle and their development thereby inhibited.

In these two experiments it was proven beyond doubt that the anaerobic bacillus isolated from sour hams was capable of producing souring when introduced into the bodies of sound hams; and in view of the fact that this bacillus was constantly present in hams which had undergone spontaneous or natural souring, and was the only organism that could be isolated from such hams that was capable of producing in egg-pork cultures the characteristic sour-ham odor, the conclusion seems justifiable that this bacillus is an undoubted cause of the ham souring which occurs in the packing house; and the results thus far obtained indicate that it is an important, if not the only, factor concerned in ham souring.

Having established the etiological relation of the bacillus isolated from sour hams with ham souring, the next point to be considered was the manner in which this bacillus finds its way into the bodies of the hams.

PROBABLE METHOD BY WHICH HAM-SOURING BACILLUS ENTERS HAMS.

Regarding the question of the probable method by which the ham-souring bacillus enters hams, there were three possibilities to be taken into consideration: (1) That the bacillus is present in the flesh of hogs at the time of slaughter, (2) that the bacillus gains entrance through the pickling fluids, (3) that the bacillus is introduced into the bodies of the hams in the handling or manipulation which the hams undergo in preparation for, or during, the process of curing.

Possibility of Infection Prior to Slaughter.

In order to throw some light upon this point, a number of fresh hams—that is, hams which had been chilled but not pumped or subjected to any other manipulation—were examined bacteriologically, but in no case could the anaerobic bacillus which was isolated from sour hams be detected in any of them. The fact that in certain of the smaller packing establishments which cure their hams without pumping the percentage of souring is extremely low would also seem to negative this possibility, for if the bacillus which causes souring were present in the hams at the time of slaughter, sour hams would be as frequent at such establishments as at those establishments which make a practice of pumping. Furthermore, a laboratory study, biological and chemical, of the bacillus isolated from sour hams shows that this organism belongs to the class of putrefactive bacteria, and while such bacteria may be present in the intestines of healthy animals, as, for example, the bacillus of Bienstock (*Bacillus putrificus*), these bacteria do not invade the organs and tissues of the body until after the death of the animal, and the packing-house practice of rapidly eviscerating the hogs immediately after slaughter would certainly preclude this possibility.

Possible Infection from Pickling Fluids.

With regard to the second possibility, that the bacillus finds its way into the hams in the curing pickles, it was determined by laboratory experiment that the addition of 3 per cent of sodium chlorid or 3 per cent of potassium nitrate to laboratory media completely inhibits the growth of the bacillus. As the pickling solutions always contain considerably more than these percentages of sodium chlorid and potassium nitrate, it would be impossible for the bacillus to multiply in the pickles. Additional laboratory experiments demonstrated, however, that the bacillus or its spores may remain alive in the curing pickles for at least thirty days, and it seemed possible that the curing pickles might become contaminated at times with the bacilli, and that the bacilli, although incapable of multiplying in the pickles, might find their way into the bodies of the hams in the pickling fluids. In order to throw some light upon this point, the following experiment was carried out:

EXPERIMENT TO SHOW WHETHER INFECTION TAKES PLACE FROM THE CURING PICKLE.

In this experiment two tierces were put down, each containing 20 hams. The hams weighed from 14 to 16 pounds and had received the usual 48-hour chilling. The pickling solutions employed were the regular curing pickles of the establishment at which the experiment was carried out. The curing pickle in one tierce was inoculated with 400 cubic centimeters of a culture suspension of the bacillus, prepared in the same manner as that used for the injection of the hams in tierce 2 in Experiment II. A microscopic preparation made from a small drop of the culture suspension before adding it to the pickle showed the bacilli in large numbers, and in the 400 cubic centimeters of the suspension there were millions of the bacteria. The curing pickle in the other tierce was left untreated, the hams in this tierce serving as a check. The tierces used in this experiment, as in all of the experiments, were thoroughly cleaned with boiling water before the hams were placed in them. The experiment was conducted in a pickling room which was held at 33° to 36° F., and the tierces were rolled three times during the cure. The details of the experiment are as follows:

Tierce 1.—Contained 20 hams, half of which were pumped in both body and shank and half in the shank only. As soon as they were pumped the hams were packed in the tierce. Sufficient curing pickle to fill the tierce was then measured out in a clean barrel and to it was added the culture suspension. The culture was thoroughly mixed with the pickle and the latter was then run into the tierce containing the hams.

Result: When tested at the end of the cure, two of the hams which had been pumped in the shank only showed slight souring in the body. The rest of the hams were sweet.

Tierce 2.—Contained 20 hams which were pumped in the same manner as those in tierce 1. The curing pickle was the same as that used for tierce 1, but without the addition of culture. This tierce was put down as a check on tierce 1, the hams being cured under exactly the same conditions, but without the addition of culture to the curing pickle.

Result: One of the hams which was pumped in the shank only developed slight souring in the body. The remainder of the hams were sweet.

Comparing tierce 1, which contained the inoculated pickle, with tierce 2, the check tierce which contained uninoculated pickle, we find there was practically no difference in the final result. In tierce 1 two of the hams developed slight souring, while in tierce 2 one of the hams became slightly sour. All of these hams had been pumped in the shank only. The fact that one of the hams in the check tierce developed slight souring was undoubtedly due to bacterial contamination in pumping or in the handling which the hams underwent prior to pickling, and the slight souring of the two hams in tierce 1 must also be attributed to the same cause or causes, for had the souring in these last hams resulted from the penetration of the bacteria from the pickling solution a higher percentage should have become sour. Furthermore, if the souring of the two hams in tierce 1 had resulted from the penetration of the bacteria from the curing pickle, the souring should have been general throughout the bodies of these hams, whereas the souring was only evident around the bone and was slight in degree.

From this experiment the conclusion would seem justified that the bacillus which causes ham souring does not usually find its way into the bodies of the hams from the curing pickle, although it would be going too far, perhaps, to say that infection never takes place from the curing pickle. The experiment, however, indicates clearly that the curing pickles are certainly not the main channel through which the hams become infected. In referring to the curing pickles, it should be understood that we refer here to the pickling solutions in which the hams are immersed, and not to the pumping pickles. The possibility of infection through the pumping pickle will be discussed later.

POSSIBLE INFECTION THROUGH MANIPULATION OR HANDLING.

There are at least three possible ways in which hams may become infected from the handling which they receive in preparation for, or during the process of curing, viz: From the thermometers used in taking the inside temperatures of the hams, from the pumping needles, and from the billhooks used in lifting the hams.

INFECTION FROM HAM THERMOMETERS.

The packing-house method of taking the temperatures of hams by means of a pointed, metal-capped thermometer which is thrust deep into the bodies of the hams has already been referred to, but deserves to be described somewhat more in detail, as it will be at once apparent that this manipulation furnishes a ready means whereby hams may become infected with putrefactive bacteria. The construction of a ham thermometer is shown in figure 4.

The instrument consists of a glass thermometer inclosed in a metal case, the front portions of the case being cut away so as to expose the scale above and the mercury bulb below. As was explained before, the thermometer is thrust deep into the body of the ham so that the pointed end containing the mercury bulb rests beside or a little behind the upper portion of the femur, the bone being used as a guide in introducing the thermometer.

Ham temperatures are taken at three stages in the preparation for cure—(1) on the hanging floor, just before the hams go to the chill rooms, in order to determine the amount of heat lost prior to chilling; (2) on leaving the chill rooms, in order to determine the thoroughness of the chill; (3) on the packing floor, just before the hams are placed in pickle, as a further check on the thoroughness of the chilling.

In taking the temperatures of hams which have been chilled—and most of the temperatures are taken subsequent to chilling—it is customary for the packing-house attendant who has this matter in charge to warm the thermometer by holding the pointed or bulb end in his hand, so as to force the mercury column up to about 60° F., or well above the temperature of hams. The thermometer is then thrust into the ham and allowed to remain for several minutes, by which time the mercury column will have fallen to the temperature of the ham. The thermometer is then slowly withdrawn so as to expose the top of the mercury column, and an accurate reading is thus obtained of the inside temperature of the ham. The thermometer is warmed by the hand before each ham is tested, and this undoubtedly insures more accurate readings than would result were the thermometer removed from one ham and plunged immediately into another, but the procedure is open to certain objections, for the open space between the metal point of the thermometer and the mercury

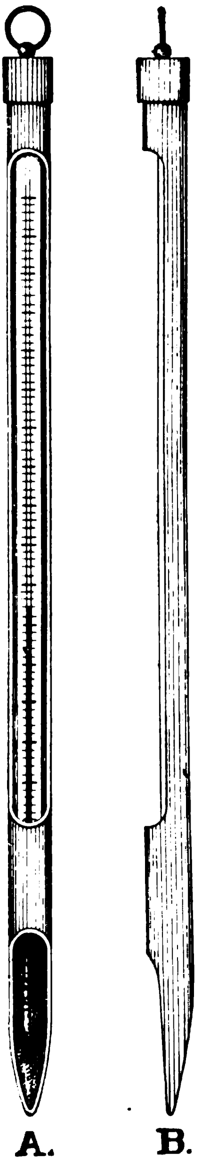


FIG. 4.—Diagrammatic views showing construction of ham thermometer. A, front view, showing open space between metal point and mercury bulb, which becomes filled with particles of meat, grease, and dirt; B, side view.

bulb soon becomes filled with particles of meat and with grease and dirt from the attendant's hands, and it is at once apparent that a thermometer in this condition would furnish a ready means whereby extraneous matter might be introduced into the bodies of the hams. In other words, a contaminated thermometer would furnish an excellent means whereby hams could be inoculated with putrefactive bacteria.

In order to determine whether hams actually become inoculated in this manner, the following experiment was carried out:

EXPERIMENT TO SHOW WHETHER HAMS BECOME INFECTED FROM HAM THERMOMETERS.

This experiment was designed to show (1) whether the usual packing-house method of taking ham temperatures was apt to induce souring in the hams thus tested, and (2) whether souring would result in hams which were tested with a thermometer purposely contaminated with the bacillus isolated from sour hams.

The experiment was carried out as follows: Thirty hog carcasses were selected as they entered the hanging floor from the killing floor. They had been cleaned, eviscerated, and split, and were of the same average weight and of sufficient size to yield hams weighing from 12 to 14 pounds. They were divided into three lots of 10 each and were allowed to remain on the hanging floor for two hours, after which they were given the usual 48-hour chilling.

Lot 1.—The hams in this lot were tested with an ordinary ham thermometer as they entered the hanging floor, as they left the hanging floor, and as they left the coolers. The thermometer used was borrowed from one of the plant attendants and was used in the condition in which it was received from him; that is, it was not cleaned or disinfected prior to use.

Lot 2.—The hams in this lot were tested as they entered the hanging floor with a thermometer which had been previously cleaned and disinfected and then dipped in a culture suspension of the meat-souring bacillus which was isolated from sour hams. The thermometer was dipped in the culture suspension before each ham was tested. No further temperatures were taken of these hams. The thermometer was carefully cleaned and disinfected before it was returned to the attendant from whom it was borrowed.

Lot 3.—The hams in this lot were not tested at all, and were intended as checks on the cure.

The three lots of carcasses were carefully tagged and were chilled in a special cooler to themselves. Upon leaving the cooler the hams were cut from the carcasses and trimmed. The three lots of hams were then cured in separate tierces. All of the hams were subjected to exactly the same cure.

The pickles used were the regular pumping and regular curing pickles of the establishment at which the experiment was carried out.

The hams in lot 3 were pumped first and those in lot 1 were pumped next. The needle was then removed and a fresh, clean needle was used for lot 2. This was done in order to prevent the possibility of carrying over bacteria from one lot of hams to another on the pumping needle. The tierces were thoroughly cleaned with boiling water before being used. The curing was carried out in a pickling cellar which was held at 33° to 36° F., the temperature never rising above the latter figure. The tierces were rolled three times during the curing. The details and results were as follows:

Tierce 1.—Contained 20 hams, half of which were pumped in both body and shank and half in the shank only. These hams were taken from the carcasses in lot 1 and had been tested several times with a ham thermometer, as already described.

Result: At the end of the cure it was found that of the 10 hams which were pumped in the shank, 5 showed well-marked souring in the body, while of the 10 hams which were pumped in both body and shank, 2 showed slight souring in the body.

Tierce 2.—Contained 20 hams, which were pumped in the same manner as those in tierce 1. These hams were taken from the carcasses in lot 2 and had been tested once with a thermometer which was dipped in a culture suspension of the bacillus isolated from sour hams.

Result: The 10 hams which were pumped in the shank only all became sour. When they were tried out at the end of the cure, they showed pronounced souring throughout the entire body and were classed as "stinkers" by the meat inspector who examined them. The souring extended through to the bone marrow of the femur in all of these hams. Of the 10 hams which were pumped in both body and shank, 7 showed well-marked souring in the body, but not as pronounced as in those pumped in the shank only; in five of these hams the souring extended through to the bone marrow of the femur, while in 2 the bone marrow remained sweet.

Tierce 3.—Contained 20 hams, which were pumped in the same manner as those in the two preceding tierces. These hams were not tested with a thermometer, and were put down as a check on the cure. They were pumped with the same pumping pickle, subjected to the same cure, and held under precisely the same conditions as the hams in the two preceding tierces.

Result: When tested at the end of the cure, all of these hams were found to be perfectly sound and sweet.

Results of experiment to show whether hams become infected from ham thermometers.

No. of tierce.	Number of hams.	Average weight of hams.	How pumped.	Treatment.	Condition at end of cure.	
					Number of sour hams.	Percentage of sour hams.
		Pounds.				
1	20	12-14	10in shank	Tested at several stages in preparation for cure with ham thermometer which had not been cleaned.	5	50
			10 in body and shank	do	2	20
2	20	12-14	10 in shank	Tested once with ham thermometer dipped in culture suspension of anaerobic bacillus isolated from sour hams.	10	100
			10 in body and shank	do	7	70
3	20	12-14	10 in shank	Not tested with thermometer.	0	0
			10 in body and shank	do	0	0

Several hams from each tierce were examined bacteriologically. cultures being taken from the meat near the bone and from the bone marrow of the femur.

In the sour hams from tierce 1 cultures taken from the meat near the bone showed the same anaerobic bacillus noted in other sour hams (i. e., the same bacillus which caused souring in Experiments I and II), but these cultures were contaminated with other bacteria which were probably introduced on the thermometer along with the ham-souring bacillus. None of the contaminating bacteria were capable, however, of producing a sour-meat odor when grown on the egg-pork medium. Pure cultures of the ham-souring bacillus were obtained from the bone marrow of some of these hams, showing that this bacillus had penetrated through to the bone marrow while the other bacteria had not.

From the sour hams in tierce 2 the ham-souring bacillus was recovered readily, and often in pure culture, from the hams which had been pumped in the shank only, whereas it was usually contaminated with pickle bacteria in the hams which had been pumped in both body and shank.

In the case of the sound hams in tierce 3, cultures taken from the meat near the bone and from the bone marrow of the femur were negative in the hams which had been pumped in the shank only, while cultures taken from corresponding points in the hams pumped in both body and shank showed ordinary pickle bacteria, which had evidently been introduced into the bodies of these hams in the pumping pickles. None of these hams exhibited the slightest sour odor.

Summary of experiment.—In this experiment 20 hams (tierce 1) were tested with an ordinary ham thermometer in the usual packing-house manner. Half of these hams were subjected to the mild cure and half were given the regular cure, with the result that 50 per cent of those receiving the mild cure and 20 per cent of those receiving the regular cure became sour.

A second lot of 20 hams (tierce 2) were tested with a thermometer which had been purposely contaminated with a culture suspension of the ham-souring bacillus. These hams were cured in the same manner as the first lot, with the result that all of those receiving the mild cure and 70 per cent of those receiving the regular cure became sour.

A third lot of 20 hams (tierce 3) which had not been tested at all were cured in the same manner as the two preceding lots, as a check on the cure. All of these hams were sweet at the end of the cure.

Inasmuch as the three lots of hams were cured under precisely the same conditions and were handled in the same manner prior to pickling, the only difference being that the hams in tierces 1 and 2 were tested with the ham thermometer while those in tierce 3 were not, we must conclude that the souring of the hams in tierces 1 and 2 resulted from the testing which these hams received. In the case of tierce 1 the hams became infected from a thermometer which, in the ordinary routine use of the packing house, had become accidentally contaminated with the ham-souring bacillus. In the case of tierce 2 the hams became infected from a thermometer which had been artificially contaminated with the bacillus. The high percentage of sours in this last lot is due to the fact that these hams were heavily infected with the ham-souring bacillus, for owing to the construction of the ham thermometer many thousands of the bacilli were unquestionably introduced into each ham on the point of the thermometer. In the ordinary routine of ham testing, where hams become infected from foreign matter introduced on the thermometer, the percentage of souring, as shown in tierce 1, would be less, for it is not to be supposed that ham thermometers are always contaminated with the ham-souring bacillus, but that they only become so at times, and that probably only a few of the bacilli are then introduced.

This experiment, we think, proves conclusively (1) that the ham-souring bacillus may be introduced into the bodies of hams on the thermometers used in testing the hams, and (2) that the packing-house method of taking ham temperatures by means of a thermometer which is thrust deep into the bodies of the hams may cause souring in the hams thus tested.

As a further proof that hams may become contaminated in this manner, a series of cultures were made from scrapings taken from ham thermometers. The scrapings consisted of the accumulations of bits of meat, grease, and dirt that collect on the thermometers, and were taken from the thermometers while the latter were in ordinary routine use in the packing house. In a series of six cultures which were made from such scrapings at different times, the same bacillus which was isolated from sour hams and shown to cause meat souring was found three times. In other words, the ham-souring bacillus was present in 50 per cent of the cultures made from thermometer scrapings, and many hams undoubtedly become infected from the thermometers. Souring would be almost certain to result in mild-cure hams if these hams were tested with a thermometer which had become accidentally contaminated with the ham-souring bacillus, as the bacillus would have time to develop within the bodies of the hams before being inhibited by the curing pickle, which penetrates slowly into the bodies of these hams. In the case of regular cure hams—that is, hams which are pumped in both body and shank—souring would be much less apt to occur after the use of a contaminated thermometer, as these hams are more or less saturated with a strong pumping pickle at the beginning of the cure, which would tend to inhibit the growth of any bacteria that might be introduced on the thermometers.

The fact that souring may result in hams from the use of a contaminated thermometer would explain the occurrence of several sours in one vat, for in testing hams just before they go into cure several hams are usually tested in succession, and these would in all likelihood go into the same vat. Supposing the thermometer to have been contaminated with the ham-souring bacillus at the time these hams were tested, this would explain a fact which has been often noted, namely, the occurrence of several sours in one vat while other vats containing the same run of hams show no sours.

If souring resulted in all of the hams which are subjected to a thermometer test in the daily routine of the packing house, this manipulation alone might account for nearly all of the sours which occur, but the experiment which has been just described shows that all of these hams do not become sour. In tierce 1, where each ham was subjected to three thermometer tests at different times, souring resulted in 35 per cent (this includes both mild and regular cure) of the hams thus tested, and in actual practice the percentage of sours in hams which have been subjected to the thermometer test would probably be somewhat less. Quite a large percentage of sour hams are thus left unaccounted for by the thermometer test, and we believe that these are chiefly the result of contamination carried in on the pumping needles or in the pumping pickles.

INFECTION FROM PUMPING NEEDLES.

In view of the results obtained in the last experiment, in which it was shown that hams may become infected from the use of ham thermometers, it seemed not improbable that hams might also become infected from the pumping needles, which, like the thermometers, are thrust deep into the bodies of the hams beside the bone. In order to throw some light upon this point, cultures were taken from the grease and dirt that accumulate on the shields at the bases of the pumping needles, as such material must undoubtedly be carried into the hams at times on the needles. The ham-souring bacillus was found several times in these cultures, and hence it is fair to infer that hams may also become infected at times from the pumping needles, just as they become infected from the thermometers. Bits of contaminated meat and grease and particles of dirt carried in on the pumping needles would be forced out into the hams by the pumping pickle, which passes out through small openings or fenestræ in the needles, and this probably affords one explanation as to why so many more body sours occur in the mild-cure hams. In the mild-cure hams, which are pumped in the shank only, the pumping needle is introduced near the femorotibial articulation, and the shank is saturated at the start with a strong brine solution, while the body of the ham is not. If the ham-souring bacillus were carried into these hams on the pumping needle, the growth of the bacillus in the shank would be inhibited by the strong brine solution with which the shank is saturated, but there would be nothing to prevent the bacillus from growing upward into the body of the ham, which has not been pumped and is free from pickle. This would also explain the fact that the souring often starts at the knee joint and extends upward into the body of the ham. In the case of the regular cure hams, where the ham is pumped in both body and shank, the entire ham is more or less saturated at the start with the strong brine of the pumping pickle, which tends to inhibit the growth of the ham-souring bacillus even if this bacillus should find its way into these hams on the pumping needles. It is in the mild-cure or partly pumped hams, where the body of the ham is left unpumped, that the ham-souring bacillus finds its best opportunity for development, and the greater proportion of the sours that occur in the packing house are found in these hams.

As regards the possibility of infection from the pumping pickle itself, it does not seem probable that this would often occur, for the pumping and curing pickles are always prepared on an upper floor of the pickling houses and are delivered to the pickle cellars in closed pipes, so the chances for the accidental contamination of these solutions from floating dust or dirt would not be great. Furthermore, the strong brine of the pumping pickle would completely inhibit the growth of the ham-souring bacillus, and the bacillus would be incapable of multiplying, even if it found its way into the pickle. On the other hand, laboratory experiments show that the bacillus or its spores may remain alive for a considerable length of time in the pumping pickle, so the possibility of infection from this source can not be overlooked.

INFECTION FROM BILLHOOKS.

After the hams are cut from the carcasses they are handled entirely by means of billhooks. In handling the hams the hooks are inserted beneath the skin of the shank at a point just above the tibio-femoral articulation. The hooks should be inserted in the connective tissue beneath the skin and should not penetrate the muscular tissue to any depth. When the hams lie in the right position, with the butt or large portion away from and the shank toward the operator, it is an easy matter to pick them up in the proper manner; but when they lie at different angles and are being rapidly handled it is almost impossible to prevent the hook from penetrating the muscular tissues, and if the hook should penetrate to the bone it might carry in foreign matter contaminated with the meat-souring bacillus. It is not probable that many hams become contaminated in this way, as the men who handle the hams are very skillful in manipulating their hooks; but the possibility that hams may become contaminated in this manner should not be entirely overlooked.

BIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF THE HAM-SOURING BACILLUS.

CONDITIONS FAVORABLE TO GROWTH.

The most favorable medium for the growth of the organism was found to be the modified egg-meat mixture of Rettger, which has been previously described. In this medium the organism develops rapidly at a temperature of 20° to 25° C., giving rise to the characteristic sour-meat odor. Like the bacillus described by Klein, it also grows readily on pork-agar and pork-bouillon containing glucose, but differs from Klein's bacillus in that it will grow, though less luxuriantly, on ordinary nutrient media—agar, gelatin, and bouillon—without the addition of glucose.

The optimum temperature for growth is 20° to 25° C. The organism does not grow at incubator temperature (37.5° C.). At ice-box temperature (8° to 10° C.) it develops readily, although the growth is less rapid than at 20° to 25° C. That the organism will develop at even lower temperatures was shown in the inoculation experiments with hams, where it developed and multiplied extensively in the bodies of the hams at the temperature of the pickling cellars, which are held usually at 34° to 36° F. (1° to 2° C.).

The organism develops best in a neutral or slightly alkaline medium.

GROWTH ON DIFFERENT CULTURE MEDIA.

Growth on egg-pork medium.—At a temperature of 20° to 25° C. the cultures show a slight but distinct sour odor in from two to three days. This odor, as before stated, closely resembles the odor of a sour ham. Egg-pork cultures from three to five days old were given to a trained meat inspector, who knew nothing whatever as to the contents of the tubes, and he was asked to describe the odor; he described it as that of a sour ham.

At one week the albumins of the medium are gelatinized or partly coagulated and the odor is more pronounced. At ten days the albumins are completely coagulated except at the surface, where there is no apparent growth; the odor is more putrefactive in nature, and the reaction of the medium is slightly acid. At three weeks the coagulated albumin splits up into fragments and appears to undergo a slow digestion, gas bubbles form in the lower portion of the culture, and the odor becomes distinctly putrefactive in character. The slow digestion of the albumin is probably due to a proteolytic enzym elaborated by the bacillus.

At the end of a week a dark zone usually appears at the surface of the coagulated albumin and gradually darkens until it becomes almost black. This zone is probably due to a pigment elaborated by the bacillus.

At ice-box temperature (8° to 10° C.) the same changes and the same odor were noted, but were somewhat slower in developing.

Glucose-pork-agar.—This medium was prepared from pork in the same manner as beef-agar, and contained 1 per cent of glucose. The organism grows readily on this medium and may be conveniently cultivated in deep stab cultures. The medium was always thoroughly boiled and then rapidly cooled in order to expel the inclosed air. The growth of the organism was found to vary considerably with the reaction.

When the reaction was +1.5, deep stab cultures at three days (20° to 25° C.) showed a well-marked aborescent growth, appearing as delicate filaments extending outward from the line of stab. The growth stopped within one-fourth or one-half inch of the surface of the agar on account of the presence of oxygen in the upper part of the culture medium. As the growth extended toward the walls of the test tube the agar became clouded, and there were sometimes gas bubbles in the depth of the agar, but the gas formation was not extensive.

When the reaction of the agar is neutral or slightly alkaline, extensive gas formation occurs and the agar is often much broken up.

The cultures developed a disagreeable, somewhat putrefactive odor, but did not give the characteristic sour-ham odor obtained from the egg-pork cultures.

The organism was also grown on anaerobic agar plates by Zinsser's method, which is said to give absolutely anaerobic conditions. The colonies on agar have a cottony or woolly appearance at first, and spread slowly, with slightly irregular margins.

In glucose-pork-agar to which azolitmin was added the azolitmin in the lower portion of deep stab cultures was completely decolorized in five days at room temperature (20° to 25° C.).

In glucose-pork-agar containing neutral red the red color in the lower portion of the tube was changed to yellow with the development of fluorescence.

Neutral gelatin.—Tubes of ordinary neutral gelatin without the addition of glucose were inoculated and held at ice-box temperature (8° to 10° C.). At five days a delicate white growth appeared along the line of stab in the lower portion of the tube. At seven days the growth showed fine radial striæ, presenting an arborescent or tree-like appearance, and extended halfway from the line of stab to the walls of the test tube. At two weeks the growth had caused a delicate clouding of the medium in the lower portion of the tube. At three weeks the gelatin in the lower portion of the tube had become liquefied and the growth had settled to the bottom as a white precipitate.

In gelatin containing glucose, gas bubbles are formed in the depth of the medium through the splitting up of the glucose, and the characteristic arborescent growth is obscured.

Glucose-pork-bouillon.—This medium was prepared from pork instead of beef and contained 1 per cent of glucose. The best results were obtained when the reaction of the medium was neutral or slightly alkaline.

Culture tubes, which had been previously boiled to expel the contained air and then inoculated, were held in a Novy jar, in an atmosphere of hydrogen at a temperature of 20° to 25° C. At three days the tubes showed well-marked clouding. At one week the growth appeared as a heavy, white, flocculent, cottony precipitate in the bottom of the tubes with a slight flocculent precipitate above. When the culture was removed from the jar and shaken, the heavy, flocculent precipitate at the bottom of the tube broke up without much difficulty, giving rise to a heavy uniform clouding with some small floating masses, which soon settled to the bottom. On shaking the tube some evolution of gas in the form of very fine bubbles was noticed.

In Smith fermentation tubes containing neutral glucose-pork-bouillon the closed arm of the tube shows well-marked clouding with gas formation at three days at room temperature (20° to 25° C.). The growth has a tufted, cottony appearance, and there are many filaments and threads. The growth settles to the bottom of the closed arm as a cottony, white precipitate (see Pl. IV). The organism splits the glucose vigorously, and at 10 days the tubes show from 40 to 50 per cent of gas. The bouillon in the open arm of the tube remains unclouded. The maximum gas production at room temperature is reached in from 10 to 14 days, by which time the growth in the closed arm has completely settled into the bend of the tube, leaving the bouillon in the closed arm clear. The gas formula, as determined by Smith's method, was $\frac{H}{CO_2} = \frac{5}{1}$. The reaction of the bouillon becomes acid to phenolphthalein.

The organism will grow on ordinary neutral bouillon without the addition of glucose, and in Smith tubes containing this medium a small amount of gas was formed, due to the splitting of the muscle sugar.

The bacillus also grows in a sugar-free broth—that is, a broth free from muscle sugar—and from cultures grown in this medium a well-marked indol test was obtained.

Litmus-milk.—The organism was grown in litmus-milk in Smith fermentation tubes at 20° to 25° C. At seven days the litmus in the lower portion of the closed arm had assumed a brownish-buff color. At two weeks the litmus in the closed arm had been reduced to a brownish-buff color except at the top of the tube, where a pale, bluish tinge remained, and the litmus in the open arm showed very slight reddening as compared with a check tube. At three weeks the litmus in the closed arm was entirely reduced to a light, brownish-buff color, and the litmus in the open arm showed a slight but distinct reddening as compared with the check. The reddening of the litmus in the open arm was evidently due to the transfusion of acids formed by the growth of the bacillus in the closed arm. After several weeks the milk is slowly peptonized, probably as a result of enzym action.

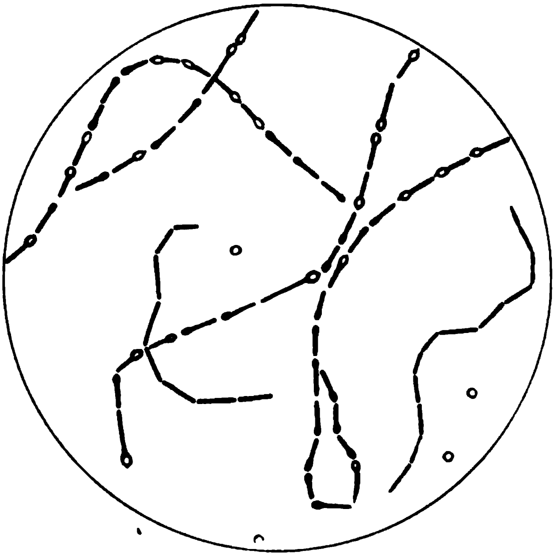


FIG. 5.—Ham-souring bacillus (*Bacillus putrefaciens*) grown on egg-pork medium, showing tendency to form chains. Partly developed and fully developed spores are shown at ends of rods; also free spores. (Pen-and-ink drawing made with camera lucida from preparation stained by Gram's method. × 640.)

MORPHOLOGY.

The organism is a large bacillus having an average size of 4 to 8 μ in length by 0.5 to 0.7 μ in thickness, but there are many longer forms measuring from 10 to 20 μ in length. It develops in long, irregular chains or filaments, which at times show a slightly spiral form.

The individual organisms show at times a widely open, slightly spiral form, which was more apparent in hanging-drop preparations made from bouillon cultures, where the organisms had been comparatively undisturbed. This appearance was also noted at times in the stained sections of soured muscular tissue, where the organisms were stained in place. The organism possesses no motility. It stains with the ordinary aniline dyes and by Gram's method.

SPORE FORMATION.

The organism develops large, terminal spores, which are at first oval, but when fully developed are perfectly round and measure from 1.5 to 2 μ in diameter.

Spores develop rapidly in the egg-pork medium at 20° to 25° C., fully developed spores being noted in from five to seven days. At ice-box temperature (8° to 10° C.) partly developed spores were noted in the egg-pork medium at 10 days and fully developed spores at 2 weeks.

Occasional spores were noted in old agar and gelatin cultures, but abundant spore formation was seen only in the egg-pork medium. No spores were noted in bouillon cultures, even at 10 weeks.

RESISTANCE TO HEAT AND CHEMICAL AGENTS.

In its vegetative form the bacillus is killed at 55° C. in 10 minutes. The spores survive a temperature of 80° C. for 20 minutes, but are killed at 100° C. in 10 minutes.

When sodium chlorid and potassium nitrate were added to glucose-pork broth in varying amounts, it was found that 3 per cent of sodium chlorid or 3 per cent of potassium nitrate was sufficient to inhibit completely the growth of the bacillus at room temperature (20° to 25° C.).

While the growth of the bacillus was inhibited by sodium chlorid and potassium nitrate as just stated, it was found that very much stronger solutions of the two salts failed to destroy the bacillus. Thus it was found that the bacillus or its spores retained their vitality after an exposure of 30 days in a solution containing 23 per cent of sodium chlorid and 6 per cent of potassium nitrate.

GAS PRODUCTION.

The organism splits glucose, but not lactose or saccharose. That it possesses the power of splitting muscle sugar was shown by the formation of gas in Smith fermentation tubes containing ordinary neutral bouillon without the addition of any sugar.

The formation of gas in glucose bouillon varies considerably with the reaction of the medium. The largest amount of gas was formed when the broth was neutral or slightly alkaline. When the reaction of the broth was distinctly acid or distinctly alkaline the amount of gas was diminished. The gas which is formed in bouillon cultures consists chiefly of hydrogen and carbon dioxid. In order to collect a sufficient amount of the gas for analysis, two large fermentation tubes capable of holding 150 cubic centimeters each were constructed. These tubes were filled with pork-bouillon and inoculated with the bacillus. After 20 days at room temperature (20° to 25° C.) the gas was collected and the carbon dioxid and hydrogen determined, with the following result:

	Cubic centimeters.
Total amount of gas collected	37. 7
Carbon dioxid, by absorption with NaOH	6. 2
Hydrogen, by difference	31. 5

This analysis gives an approximate gas formula of $\frac{H}{CO_2}=\frac{5}{1}$, which agrees with the gas formula as determined in the small fermentation tubes by Smith's method.

In hams which had undergone spontaneous souring and in hams which had been artificially soured by inoculation, hydrogen-sulphid was often noted when the sour portions of the meat were tested with lead-acetate paper, but no distinct odor of the gas could be obtained. Hydrogen sulphid was also noted in egg-pork cultures of the bacillus.

ACID PRODUCTION.

In glucose-bouillon, butyric and lactic acids are formed and the reaction of the medium becomes distinctly acid. Butyric and lactic acids were also noted in the egg-pork cultures.

A series of Smith fermentation tubes containing 10 c. c. each of glucose-pork broth medium was inoculated with the bacillus and held at room temperature (20° to 25° C.). These cultures were titrated against $\frac{N}{40}$NaOH, with phenolphthalein as an indicator at intervals of two days up to nineteen days, and then at two-week intervals up to sixty-one days. Three of the cultures were titrated each time so as to give a fair average of the acidity of the cultures, and an uninoculated check tube was also titrated each time to see if there was any change in the reaction of the medium. The results of the titrations are shown in the following table:

Acidity determinations in glucose-pork broth cultures.

Age of culture (days).	Culture A.	Culture B.	Culture C.	Average.	Medium.	Acidity of culture.
						Per cent.
2	0.038	0.030	0.040	0.036	0.009	0.027
4	.105	.100	.102	.102	.009	.093
6	.106	.110	.109	.108	.009	.099
8	.124	.115	.117	.119	.009	.110
10	.128	.130	.126	.128	.009	.119
12	.129	.120	.129	.126	.009	.117
19	.126	.125	.125	.125	.009	.116
33	.125	.123	.125	.124	.009	.115
47	.122	.120	.121	.121	.009	.112
61	.121	.116	.119	.118	.009	.109

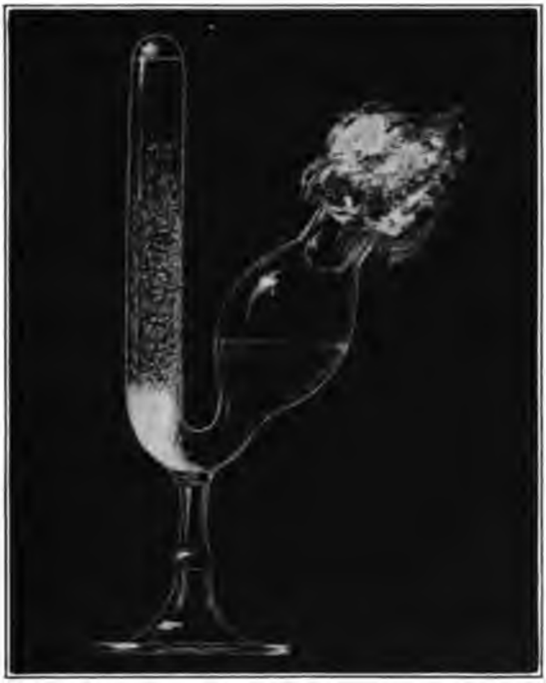
From the above table it will be seen that the maximum acidity was reached at ten days, after which there was a gradual reduction in the acidity, due probably to the formation of ammonia compounds.

PATHOGENIC PROPERTIES.

Rabbits, guinea pigs, and white mice were inoculated and fed with cultures of the bacillus without effect, from which it would appear that the bacillus possesses no pathogenic or disease-producing properties.

NATURE OF THE BACILLUS.

The bacillus is essentially a saprogenic bacterium with zymogenic properties. A preliminary study of the chemical changes which take



GLUCOSE BOUILLON CULTURE IN SMITH FERMENTATION TUBE AT FOUR DAYS. CULTURE GROWN AT ROOM TEMPERATURE (20° TO 25° C.). GROWTH CONFINED ENTIRELY TO CLOSED ARM, WITH GAS COLLECTING AT TOP.

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place in sour hams shows that these changes are of a putrefactive nature. Hams which had undergone spontaneous souring were compared with hams which had been artificially soured by inoculation, and the chemical changes were found to be identical. A chemical study was also made of the changes taking place in egg-pork cultures of the bacillus at different stages of growth, and these changes were found to be of a putrefactive nature and similar in character to the changes which occur in sour hams. Among the putrefactive products formed by the growth of the bacillus in the egg-pork medium were indol, skatol, volatile fatty acids, skatol-carbonic acid, and hydrogen sulphid.[1]

A more extended study is now being carried on in the Biochemic Division of the chemical changes which take place in hams during the process of souring, together with a further study of the chemical changes which result from the growth of the bacillus in the egg-pork medium. The results of this investigation will be given in a later paper.

The bacillus described in this paper belongs to the class of putrefactive anaerobes, which are widely distributed in nature in dust, soil, and excrementitious matters. This group of bacteria contains both pathogenic and nonpathogenic forms. The former have received considerable attention, but the latter have never been thoroughly cleared up. The bacillus isolated from sour hams belongs in the latter category, being possessed of no pathogenic or disease-producing properties. It occurs in the dust and dirt of the packing house and finds its way into the hams in the various manipulations to which the hams are subjected.

The bacillus described in this paper does not seem to correspond with any forms heretofore described. It differs from Klein's bacillus (*Bacillus fœdans*) in the following important particulars: (1) It forms large terminal spores, whereas Klein's bacillus formed no spores; (2) it will grow at a temperature of 34° F., while Klein's bacillus did not grow below 50° F.; (3) it produces an acid reaction in culture media, while Klein's bacillus gave a distinctly alkaline reaction; (4) it will grow on the ordinary nutrient media—gelatin, agar, and broth—without the addition of glucose, while Klein's bacillus did not; (5) it peptonizes the casein in milk, whereas Klein's bacillus had no action on milk; (6) it liquefies gelatin more rapidly, causing complete liquefaction after three weeks at 8° to 10° C., whereas Klein's bacillus caused only partial liquefaction after eight weeks at 20° C.; (7) it can be conveyed from turbid broth cultures to new culture material by means of the platinum loop, whereas Klein's bacillus could not be thus conveyed.

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[1] The tests for the putrefactive products formed by the growth of the bacillus in the egg-pork medium were made by P. Castleman, of the Biochemic Division, who also determined the percentage composition of the gas formed by the growth of the bacillus in the glucose-pork-bouillon medium.

For the bacillus described in the present paper the following name is proposed: *Bacillus putrefaciens.*

PREVENTION OF HAM SOURING.

As it has been shown that souring in hams results from the growth of a bacterium which is introduced into the bodies of the hams in the various manipulations which the hams undergo, the only way to eliminate souring in hams, as they are cured in the larger packing establishments, would be to cure the hams under aseptic or sterile conditions, which would, of course, be a physical impossibility.

While it will probably be impossible, therefore, to eliminate souring entirely under the methods of ham curing which are at present employed in the larger packing establishments, much can undoubtedly be done toward reducing the percentage of sours. In the matter of taking ham temperatures, for instance, if the thermometers used were thoroughly cleaned and disinfected and the surfaces of the hams seared at the point where the thermometer is introduced, infection from this source could be entirely prevented; or it might be possible so to regulate the temperature of the chill rooms that the taking of ham temperatures could be discontinued.

The elimination of the souring that results from the introduction of foreign matter on the pumping needles could be effected in two ways only, (1) by not pumping the hams at all, or (2) by pumping them under sterile or aseptic conditions. As has been stated before, some of the smaller packing establishments cure their hams without pumping, and in these establishments the percentage of sours runs very low. When hams are cured without pumping, however, the period of curing has to be materially lengthened in order to give the curing pickles sufficient time to penetrate thoroughly, and this is what the larger plants wish to avoid because of the greater space and greater number of vats which would be necessitated. The object of pumping in the larger plants, where the number of hams handled daily runs into the thousands, is to hasten the cure and thus prevent the accumulation of a great number of hams at one time. It is doubtful, therefore, whether the larger packing houses could conveniently discontinue pumping.

To pump the hams under aseptic conditions would necessitate a technique far too elaborate for routine use in the packing house; in fact, anything like complete asepsis would be out of the question. Certain measures might be adopted, however, that would tend to prevent the possible introduction of ham-souring bacilli in the process of pumping. It would undoubtedly be safer, for instance, to boil the pumping pickle before use, and the chances of carrying in contaminated foreign matter on the pumping needles could be lessened by sterilizing the pumps and needles with boiling water and by frequently dipping the needles, while in use, in boiling water. If the hams were sprayed with clean water just prior to pumping, there would be less likelihood of carrying in foreign matter on the needles. The danger of introducing contaminated foreign matter on the needles might be further obviated by searing the surfaces of the hams at the points where the needles are introduced; but such a procedure would be hardly practicable in the larger packing houses, where the great number of hams cured necessitates rapid handling.

While the danger of possible contamination in pumping, through the introduction of contaminated foreign matter on the pumping needles, can not well be avoided, this danger is partly counterbalanced by the inhibitory action of the pumping pickle, which is strikingly shown in the experiments which have been described. In these experiments, 100 hams received large doses of the ham-souring bacillus, half of these hams being subjected to the mild cure and half to the regular cure, with the following result: In the case of the mild-cure hams, which were pumped in the shank only, the percentage of sours was practically 100 per cent, every ham with possibly one exception becoming sour; whereas in the regular-cure hams, which were pumped in both body and shank, only 58 per cent of the hams became sour. In other words, the additional pumping which the regular-cure hams received served to prevent souring in 42 per cent of these hams. In these experiments the number of bacteria introduced into the hams was very great, thousands and even millions of the bacilli being introduced into each ham, whereas in the routine of the packing house it is not likely that more than a few of the bacilli are ever introduced at one time on the thermometers and pumping needles. In view of these results it is safe to say that in the larger packing houses, where pumping seems to be necessary, the number of sours could be reduced fully 50 per cent if all hams were pumped in the body as well as in the shank.

At present the usual procedure is to pump all hams, both mild and regular cure, with the same pumping pickle, the mild-cure hams being pumped in the shank only and the regular-cure hams at two additional points in the body. The experiments quoted above show that the additional pumping which the regular-cure hams receive undoubtedly tends to prevent the development of souring in these hams, and this result is unquestionably due to the inhibitory action of the salts contained in the pumping pickle, as it was found by laboratory experiment that the addition of 3 per cent of sodium chlorid to culture media is sufficient to inhibit the growth of the ham-souring bacillus. The pumping pickles consist of strong brine solutions and always contain considerably more than 3 per cent of sodium chlorid. If, therefore, the pumping of regular-cure hams were made more thorough than at present, and all of the deeper portions of the ham were thoroughly saturated with the strong brine solution, souring could be largely eliminated, if not entirely prevented, in these hams, as an unfavorable medium or soil would thus be created in which the ham-souring bacillus could not develop. The ham-souring bacillus is able to develop within the bodies of the regular-cure hams because the pumping of these hams is not always thorough and there are certain areas in the inner or deeper portions of the hams in which the tissues are not thoroughly saturated with the pumping pickle.

Under the present methods of curing, the greater proportion of the sours occur among the partly pumped or mild-cure hams. These hams are pumped in the shank only, and the growth of the ham-souring bacillus within the bodies of these hams is not interfered with until the curing pickle has penetrated from the outside. As it requires several weeks for the curing pickle to penetrate thoroughly into the deeper portions of these hams, the bacillus is thus afforded a considerable interval in which to develop before it is exposed to the inhibitory action of the pickle. If these hams could be thoroughly pumped in the body at the beginning of the cure in the same manner as the regular-cure hams, the chief loss from ham souring would be eliminated. It would not do, however, to pump these hams in the body with the same pumping pickle used in the regular cure, as the meat would be rendered too salty and the mild flavor of the ham would be lost. There is undoubtedly a demand for mild-cure hams, otherwise they would not be on the market; and the question then arises how to pump these hams and still retain a mild cure. This might be accomplished by pumping these hams with their own curing pickle, which is usually a milder pickle than that employed in the regular cure, or an even milder pumping pickle might be used. If mild-cure hams were pumped in this way, the percentage of souring in these hams could undoubtedly be greatly diminished without materially affecting the flavor of the ham.

To recapitulate briefly, the prevention of ham souring is to be sought in two ways: (1) Through greater care in handling the hams and the adoption of precautionary measures to prevent the introduction of the ham-souring bacillus into the bodies of the hams, and (2) through more thorough pumping of the deeper or inner portions of the hams, so as to create an unfavorable soil or medium in which the ham-souring bacillus can not develop even if it should gain entrance into the bodies of the hams.

From what has been said it will be apparent that ham souring can probably never be entirely eliminated from the packing house under the present methods of curing, but the adoption of precautionary measures in testing and pumping hams, together with a more thorough pumping of all hams in ways similar to those suggested, would unquestionably reduce very materially the losses from this source.

GENERAL SUMMARY AND CONCLUSIONS.

1. In this paper it has been shown that ham souring, as encountered in the wet cure where the hams are entirely submerged in pickling fluids, is due to the growth of an anaerobic bacillus within the bodies of the hams. This bacillus (*B. putrefaciens*) was found in sour hams obtained from four different packing establishments. It was isolated and grown in various laboratory media, in one of which, the egg-pork medium, it gave rise to the characteristic sour-ham odor. This bacillus was the only organism that could be isolated from sour hams that was capable of producing the characteristic sour-ham odor in the egg-pork medium.

2. When injected into the bodies of sound hams, the bacillus caused these hams to sour in the process of curing. In hams which had been inoculated with the bacillus and thus artificially soured, the bacillus was recovered in cultures taken at points far removed, relatively speaking, from the point of inoculation, indicating that the bacillus had multiplied and progressed by extension throughout the bodies of the hams.

3. The bacillus possesses no motility, and its extension throughout the bodies of the hams is a result of multiplication. In its growth it follows along the connective-tissue bands between the muscle bundles, which are composed of comparatively loose tissue and afford paths of least resistance. When it invades the muscle tissue proper, it follows along the sarcolemma sheaths between the muscle fibers. As a result of this growth the muscular tissue becomes softer and tends to break more easily.

4. The bacillus belongs to the class of putrefactive anaerobes which are widely distributed in nature in dust, soil, and excrementitious matters. The bacillus or its spores is present in the dust and dirt of packing houses and finds its way into the hams in the various manipulations to which they are subjected.

5. The bacillus or its spores may be introduced into hams on the thermometers used in testing the hams, on the pumping needles, and possibly on the billhooks used in handling the hams. It may also be carried into the hams in the pumping pickle, and may even find its way into the hams from the curing pickle, although infection through the latter channel probably does not often occur.

6. The bacillus develops in the deeper portions of the ham because of the anaerobic conditions there prevailing, and souring is most often encountered, therefore, in the deeper portions of the ham near the bone.

7. A preliminary study of the chemical changes which take place in the process of souring shows that these changes are of a putrefactive nature, and ham souring, as ordinarily encountered, is to be regarded as an incipient putrefaction. Hams which had been artificially soured by injections of culture were compared with sour hams obtained from the packing house, and the putrefactive changes were found to be identical.

8. Hams which have once become sour can never be restored to a sound condition, because of the chemical changes which result from the growth of the bacillus. In other words, the tissues of the ham undergo certain chemical changes in the process of souring, and when these changes have once taken place the tissues can never be restored to a sound condition. The repumping of slightly soured hams with a strong pumping pickle will check further souring, by inhibiting the growth of the bacillus, but will not restore to a sound condition those portions of the ham which have become sour.

9. The salts of the pickling fluids have a marked inhibitory action on the ham-souring bacillus, and sours occur less frequently in regular-cure hams.

10. In regular-cure hams the growth of the ham-souring bacillus is restricted and often completely inhibited as a result of the additional pumping which these hams receive, whereby they are more or less saturated with pickle at the beginning of the cure.

11. If the pumping of regular-cure hams were more thorough and all of the deeper portions of the ham were thoroughly saturated with the pumping pickle, souring could be largely eliminated if not entirely prevented in the hams, as an unfavorable medium or soil would thus be created, in which the ham-souring bacillus could not develop. The reason that souring does develop in regular-cure hams is because the pumping is not always thorough and there are certain areas in the deeper portions of these hams which are not saturated with the pumping pickle.

12. Under the present methods of curing, the partly pumped or mild-cure hams furnish the greater proportion of the sours, as these hams are not pumped in the body and the growth of the ham-souring bacillus within the bodies of these hams is not interfered with until the curing pickle has penetrated from the outside. As it requires several weeks for the curing pickle to penetrate thoroughly into the deeper portions of these hams, the bacillus is thus afforded a considerable interval in which to develop.

13. The percentage of souring in the mild-cure hams could be greatly reduced without materially affecting the cure by pumping these hams with their own curing pickle, which is usually a milder pickle than that employed in the regular cure; and if the pumping

were thorough the number of sours in these hams could be reduced to a small figure.

14. The only way by which ham souring could be entirely eliminated from the larger packing establishments under the present methods of curing would be to handle the hams throughout under aseptic conditions, and this, for obvious reasons, would be an impossibility. The losses from ham souring may be materially reduced, however, by greater care in handling the hams and the adoption of precautionary measures designed to prevent the introduction of contaminated foreign matter into the bodies of the hams, together with more thorough methods of pumping.

ACKNOWLEDGMENTS.

In conclusion, the writer desires to express his obligations to Dr. S. E. Bennett, of the Inspection Division, inspector in charge at Chicago, for the assignment of trained meat inspectors to assist in the work, as well as for kind assistance in obtaining data and material for laboratory study, and to Dr. L. E. Day, of the Pathological Division, who kindly prepared the sections which are figured and described in the present article.

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Issued April 1, 1911.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 133.
A. D. MELVIN, CHIEF OF BUREAU.

THE DETERMINATION OF NICOTIN IN NICOTIN SOLUTIONS AND TOBACCO EXTRACTS

BY

ROBERT M. CHAPIN,
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WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., January 11, 1911.

Sir: I have the honor to transmit herewith a paper on "The Determination of Nicotin in Nicotin Solutions and Tobacco Extracts" by Mr. Robert M. Chapin, Senior Biochemist in the Biochemic Division of this bureau, and to recommend its publication in the bulletin series of the bureau.

This paper describes an accurate and rapid method for the determination of nicotin, the method being applicable especially to the examination of nicotin solutions and tobacco extracts which are used in the official dipping of sheep and cattle for scabies. The method presented by Mr. Chapin has many advantages over the Kissling method which is now generally employed, and it is believed that the paper will be of interest and value not only to Federal and State officers who have to deal with nicotin solutions, but also to manufacturers who are endeavoring to produce standard and reliable articles.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

Introductory.......... 5

The Kissling method.......... 6

The petroleum ether method.......... 8

The silicotungstate method.......... 10

  Development of the quantitative method.......... 12

  Analytical results obtained by the silicotungstate method.......... 14

  The determination of nicotin in commercial preparations.......... 15

  The effect of extraneous bases.......... 16

  Tests on a rapid modification of the method.......... 17

The steam distillation of nicotin preparations.......... 20

Summary and codified directions for routine work.......... 21

THE DETERMINATION OF NICOTIN IN NICOTIN SOLUTIONS AND TOBACCO EXTRACTS.

INTRODUCTORY.

Nicotin has long been regarded as a valuable agent for the extermination of certain species of insects infesting plants and animals. For several years proprietary nicotin solutions and tobacco extracts have been permitted by the Bureau of Animal Industry[1] for use in the official dipping of cattle and sheep affected with or exposed to scabies. Nicotin baths employed in official dipping are required to possess a minimum strength of either 0.05 or 0.07 of 1 per cent of nicotin, according to circumstances. Upon the Biochemic Division of the Bureau of Animal Industry has accordingly devolved the task of examining a considerable number of such preparations, including both samples from manufacturers who have desired to obtain permission for the use of their products in official dipping, and samples of dips actually put to such use, transmitted by bureau inspectors in the field.

The method hitherto employed by the bureau for the determination of nicotin is the Kissling method, adopted several years ago by the Association of Official Agricultural Chemists as the standard method for nicotin in tobacco and tobacco products. The Kissling method in experienced hands and in the absence of certain interfering substances unquestionably yields accurate results. But it contains a number of pitfalls for the unwary; it is cumbersome, tedious, and expensive. The bureau is now in possession of a method which appears to yield results of a higher degree of accuracy than the classical Kissling method, at a less expenditure of time, chemicals, and expensive apparatus, and to yield such results in the hands of any competent chemist of ordinary training. Therefore this paper is offered in order that the method may be available to manufacturers, who are naturally interested in maintaining their preparations for use in official dipping at the standard required by the bureau, as well as to the considerable number of manufacturing, State, and Government chemists who are concerned with the examination of nicotin preparations used as general insecticides upon both animals and plants.[1]

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[1] Bureau Animal Industry Order 143, Regulations 25 and 33, and Amendment 1.

THE KISSLING METHOD.

The Kissling method as adopted by the Association of Official Agricultural Chemists is carried out as follows:[2]

> Weigh from 5 to 6 grams of tobacco extract, or 20 grams of finely powdered tobacco, which has been previously dried at 60° C., so as to allow it to be powdered, into a small beaker. Add 10 c. c. of the alcohol-soda solution [6 grams NaOH, 40 c. c. water, 60 c. c. of 90 per cent alcohol] and follow, in the case of the tobacco extract, with enough chemically pure powdered calcium carbonate to form a moist but not lumpy mass. Mix the whole thoroughly. Transfer this to a Soxhlet extractor and exhaust for about five hours with ether. Evaporate the ether at a low temperature by holding over the steam bath, and take up the residue with 50 c. c. of the dilute sodium hydroxid solution [4 grams NaOH, 1000 c. c. water]. Transfer this residue by means of water to a Kjeldahl flask, capable of holding about 500 c. c., and distil in a current of steam, using a condenser through which water is flowing rapidly. Use a three-bend outflow tube, a few pieces of pumice, and a small piece of paraffin, to prevent bumping and frothing. Continue the distillation until all the nicotin has passed over, the distillate usually varying from 400 to 500 c. c. When the distillation is complete, only about 15 c. c. of the liquid should remain in the distillation flask. Titrate the distillate with standard sulphuric acid, using phenacetolin or cochineal as indicator. One molecule of sulphuric acid is equivalent to two molecules of nicotin.

The Kissling method involves two series of operations, (1) extraction with ether, (2) distillation with steam and titration. For convenience of discussion these two series of operations will be considered in reverse order.

Titration and steam distillation.—None of the indicators tried in this laboratory have given perfect satisfaction in the titration of distillates from commercial nicotin preparations, although some show a sharp enough change of color when used with solutions of pure nicotin. In practice, as noted by other investigators, the end point is apt to be rather indefinite, requiring some care and experience on the part of the operator if accurate results are to be obtained. A freshly prepared alcoholic solution of phenacetolin has yielded the best results in this laboratory, the practice here having been to distil over the nicotin into a measured amount of standard sulphuric acid, and to titrate back the excess of acid.

The chief drawback to the operation, however, is the fact that it reckons as nicotin all alkaline substances which may gain access to the receiver during steam distillation: Alkali extracted from the glass condenser, if such is used; alkali entrained by the current of steam; ammonia, amins, and pyridin bases, if such were present in the original extract and not removed during preceding operations; ammonia from the decomposition of proteid matter of the extract which may have reached the distillation flask; all are possible sources of error which may cause too high results to be obtained. Disregarding minor sources of error, it is certain that all ammonia, amins, and pyridin bases must be eliminated before the distillation with steam is undertaken. This end is not accomplished by the evaporation at a low temperature over the steam bath directed for the removal of ether, for experiments in this and other laboratories have shown that such evaporation must cease while there is still a considerable quantity of ether remaining over the alcoholic-aqueous residue in the extraction flask or loss of nicotin will occur.

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[1] In the issue of the Chemiker-Zeitung of January 10, 1911 (Jahrgang XXXV, nummer 4, seite 30), in an article entitled "Zur Bestimmung des Nicotins in konzentrierten Tabaksäften," Dr. Johannes Schröder reviews the most recent work published upon the determination of nicotin, notes some inadequacies in methods at present in vogue and forcibly presents the pressing need for a method which, being accurate, practical, and universally employed, shall constitute a standard method for the valuation of a class of preparations which, according to figures cited, are employed in large quantities.

[2] U. S. Department of Agriculture, Bureau of Chemistry Bulletin 107, Revised 1910.

Extraction with ether.—From the preceding it is clear that if volatile alkalis other than nicotin are to be eliminated at all during the Kissling process it must be during the operation of extraction with ether. That such is actually the case may be plainly shown by a slip of litmus paper inserted in the top of the condenser during extraction. The official directions of the Association of Official Agricultural Chemists prescribe an extraction time of five hours, which period is undoubtedly sufficient to extract all the nicotin. It is not, however, in many cases nearly long enough to volatilize all ammonia and amins, particularly if the condenser used is of the spiral form from which evolved vapors can not find a ready escape. The process of extraction must be continued until a strip of red litmus paper inserted in the upper end of the condenser retains its color permanently. The length of time required for such a complete elimination of extraneous volatile bases is naturally very variable, but an extraction period of more than 15 hours has sometimes proved necessary in this laboratory. It is to be noted that in this way only ammonia and amins are eliminated. Pyridin bases, on account of their high boiling points and great solubility in ether, remain behind with the nicotin.

The Kissling method, therefore, may yield accurate results, but it yields them only in the absence of certain interfering substances and only in the hands of a chemist of special experience. It is, moreover, highly probable that in the case of commercial nicotin preparations the apparently accurate results obtained are in reality simply a nice balancing of errors. In the necessary manipulation and during the long period of extraction often required, a considerable amount of ether is lost. Bertrand and Javilier state that their experiments show that in the distillation of ethereal solutions of nicotin, between 1 and 2 milligrams of the alkaloid accompany each 100 c. c. of ether distilled, the loss becoming still more notable in the case of fairly concentrated solutions. In a properly executed determination by the Kissling method the inevitable loss of small amounts of nicotin is probably fairly well balanced by the equally inevitable retention of small amounts of ammonia and amins.

Aside from the question of accuracy, other factors, such as the amount of personal attention required, the employment of expensive apparatus, and the considerable loss of ether which inevitably occurs, contribute to make the process an expensive one, particularly when in view of the rather loose agreement between check determinations four or five repetitions may be necessary in order to obtain a thoroughly substantiated result.

THE PETROLEUM ETHER METHOD.

About 10 years ago Toth[1] employed a method for the determination of nicotin in tobacco, which appeared to give good results. The tobacco leaves were rubbed up in a porcelain mortar with a small quantity of strong caustic-soda solution, then thoroughly incorporated with plaster of Paris until a dry powder resulted. The latter was brought into a stoppered glass cylinder and thoroughly shaken with a definite volume of a mixture of petroleum ether and ethyl ether. After subsidence an aliquot of the ether was pipetted out, treated with indicator and excess of standard acid, and finally titrated back with standard alkali. Toth found that when ammonium salts were present only a very little ammonia was dissolved by the ether, and that the trace so dissolved could be readily removed by blowing air through the ether for a short time, though with some danger of simultaneous loss of nicotin.

In searching for an improvement on the Kissling method first attempts were directed toward a modification of Toth's method along the following lines:

To a weighed amount of nicotin solution or tobacco extract was added about 4 c. c. of kerosene, followed by a few grams of calcium hydrate or a very few cubic centimeters of a nearly saturated caustic soda solution. After thorough mixing, plaster of Paris, previously moistened with kerosene, was added in liberal excess of the quantity needed to combine with all water present, and the mass was mixed to uniform consistency. During the process of dehydration the kerosene previously added would presumably act as a solvent for liberated nicotin and so prevent loss by volatilization. The mixture was then transferred to the capsule of a Soxhlet apparatus and extracted with petroleum ether. On account of the total absence of water and the slight degree of solubility of ammonia and amins in petroleum ether, these substances were rapidly evolved from the top of the condenser, and a slip of red litmus paper placed therein retained its color after a very much shorter period of extraction than is required in the Kissling method.

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[1] Toth, Julius. Neue Methode zur Bestimmung des Nicotins im Tabak und in den wässerigen Auszügen der Tabakblätter. Chemiker-Zeitung, Jahrgang 25, No. 57, p. 610, July 17, 1901.

The petroleum ether extract remaining in the extraction flask was transferred to a separatory funnel and shaken out with a measured quantity of standard acid, followed by two or three washings of water. The excess of acid in the whole aqueous extract was lastly titrated back with standard alkali, following the method formerly used by Loesch.

The following table will illustrate the character of the results obtainable by this method:

TABLE 1.—*Comparative results of Kissling and petroleum ether methods on commercial nicotin solutions and tobacco extracts.*

Character of material.	Kissling method.	Petroleum ether method.
Tobacco extract, No. 754	2.92 2.88	2.98 2.92 2.96
Nicotin solution, No. 755	39.80 40.03 40.75	39.80 40.45
Nicotin solution, No. 810	38.89 39.54 39.78 39.42	40.51 40.08 40.42
Tobacco extract, No. 834	2.91 2.84	2.98 2.88 3.03
Nicotin solution, No. 835	41.40 40.84 40.62 40.29	40.70 40.91
Nicotin solution, No. 841	40.29 39.86	40.49

The method appears, therefore, to yield results which check very well with those obtained by the Kissling method. Its positive advantages are (1) much saving of time and expense by the replacement of a long period of extraction with ethyl ether by a short period with petroleum ether, and (2) elimination of the operation of steam distillation. But the proper preparation of the sample for extraction requires considerable manipulation, and the method is still a volumetric one, involving the use of a somewhat indefinite end point. Moreover, the presence of pyridin bases will lead to false results exactly as in the case of the Kissling method, and in addition a new source of error resulting from the possible presence of certain nonvolatile alkaloids is introduced. Hence the method described has been but little used in practice.

Incidentally Table 1 may serve to illustrate the rather wide variation between check determinations by the Kissling method when applied to commercial preparations. A difference in duplicate results of 1 per cent of the amount of nicotin operated upon must be expected to occur regularly in routine work, while a difference of 2 per cent is not unusual.

THE SILICOTUNGSTATE METHOD.

In 1909 Bertrand and Javillier[1] published a paper on the use of silicotungstic acid as an aid in the quantitative determination of nicotin, employing it in the examination of tobacco. The first steps in their method were to extract nicotin by boiling the tobacco leaves with dilute hydrochloric acid, then to precipitate the nicotin by the addition of silicotungstic acid. The precipitate of nicotin silicotungstate, after washing, was brought into a flask, and the nicotin, liberated by an addition of calcined magnesia, was distilled over with steam, and determined in the distillate by titration with standard acid.

The method resembles the Kissling method to the extent that the nicotin is finally determined by titration after distillation with steam, and accordingly it is subject to several similar possible inaccuracies. It successfully eliminates the effect of ammonia present as such or as ammonium salts, for ammonium silicotungstate is soluble in water. But silicotungstic acid, like some other reagents for alkaloids, is also a precipitant for proteids, and hence the nicotin silicotungstate, when brought into the flask for steam distillation, may be largely contaminated with proteid matter. It is well known that proteid matter when distilled with steam at atmospheric pressure and in the presence of caustic alkalies may yield notable quantities of ammonia. The authors therefore employ calcined magnesia at this stage of their process.

It is true that magnesia has in the past received considerable recognition as the alkali to be employed when preexisting ammonia in organic matter is to be determined. In but comparatively few cases, however, has it yielded results above suspicion, and among chemists of the present day it appears quite generally recognized that proteid matter in general is subject to a certain amount of decomposition when distilled with magnesium oxid at atmospheric pressure, so that the substance is employed in such circumstances only provisionally for lack of a more convenient method, or when results of a comparative, rather than of an absolute, degree of accuracy are required.

On the whole, then, the method as employed by Bertrand and Javillier did not appear to embody everything desired by the bureau in a method worthy to replace the Kissling method, when applied to the examination of commercial nicotin preparations.

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[1] Bertrand, G., and Javillier, M. Sur le silicotungstate de nicotine et sur le dosage de cet alcaloïde. Bulletin des Sciences Pharmacologiques, tome 16, No. 1, pp. 7–14, Jan., 1909.

At the conclusion of their paper Bertrand and Javillier append the statement that in place of the volumetric method, which they prefer on account of its greater convenience, one may substitute a gravimetric method, in which the distilled nicotin is again precipitated with silicotungstic acid, the precipitate filtered, washed, and finally ignited. From the residue of SiO_2 and WO_3 may be calculated the weight of nicotin originally present. The authors quote no experimental work to show that this process is of more than theoretical applicability; nevertheless it appeared plausible and well worth investigating. Preliminary experiments afforded encouraging results, and a thorough study of the matter was undertaken.

Silicotungstic acid is a white to yellowish-white crystalline substance, presumably of the formula $4H_2O \cdot SiO_2 \cdot 12\ WO_3 + 22H_2O$. When ignited it should leave, therefore, 85.87 per cent of a residue consisting of SiO_2 and WO_3. Ignition of 1.5397 grams of the material employed in this laboratory gave a residue of 1.3981 grams, equal to 90.08 per cent. The acid itself, and its salts with the alkalis and ammonia, are readily soluble in acidulated water.

According to Bertrand and Javillier, nicotin silicotungstate dried at a temperature of 30° C. is a rose-white salt of the formula $2C_{10}H_{14}N_2 \cdot 2H_2O \cdot SiO_2 \cdot 12WO_3 + 5H_2O$, which loses its five molecules of water of crystallization when maintained for an hour at 120° C. The solubility of the hydrated salt in water has not been definitely determined, and, at any rate, it is very probably affected somewhat by the presence of acids and salts. The above authors, however, cite the limits at which a solution of the acid may serve as a qualitative test for nicotin. At a dilution of 1 in 300,000 of nicotin in the presence of one-tenth of 1 per cent concentrated hydrochloric acid, an almost immediate opalescence follows the addition of the reagent; less or more acid prevents an immediate visible reaction, though at 1 in 200,000 it appears in presence of four-tenths of 1 per cent of acid. These limits, however, are far from marking the true solubility of the salt, for at considerably greater dilutions and in presence of much more acid it will deposit on standing, not then as a cloud or an opalescence, but in definite crystalline form. The above-mentioned authors have obtained such crystals at a dilution of 1 in 1,000,000, but have not pushed their investigations farther.

Silicotungstic acid does not form such insoluble precipitates with all alkaloids, as indicated by recent work of Javillier.[1] Conin, for example, which is of interest in this connection for the reason that, like nicotin, it is volatile with steam, yields no precipitate with silicotungstic acid at dilutions greater than 1 in 5,000.

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[1] Javillier, M. Sur les silicotungstates de conicine, de spartéine et d'atropine. Bulletin des Sciences Pharmacologiques, tome 17, No. 6, pp. 315–320, June, 1910.

In the case of some alkaloidal reagents, such as picric acid, a considerable quantity of the reagent must be present before any reaction is evident. Such appears not to be true of silicotungstic acid, for the present writer found that a 1 in 10,000 solution in one-tenth per cent hydrochloric acid yields an immediate opalescence upon the addition of one drop of a two-tenths per cent nicotin solution, increased to a heavy turbidity upon the addition of more nicotin. Hence nicotin is an appropriate reagent for the qualitative detection of small quantities of silicotungstic acid.

DEVELOPMENT OF THE QUANTITATIVE METHOD.

From the above observations it is evident that in quantitative work nicotin silicotungstate should be precipitated from a solution containing not less than one-tenth of 1 per cent of concentrated hydrochloric acid; that the precipitate should be allowed to stand before filtration until it has changed to a crystalline form; that the precipitate during filtration should be washed with water containing one-tenth of 1 per cent concentrated hydrochloric acid, and washing continued until the filtrate shows no opalescence when treated with a few drops of a dilute nicotin solution.

Since ammonia and amins are not precipitated by silicotungstic acid, the first step in a quantitative method for the determination of nicotin appeared to be the distillation of the solution or extract in a current of steam after addition of caustic soda. An aliquot of the distillate might then be precipitated with silicotungstic acid, and filtered and washed with the precautions noted above. The precipitate might then be ignited and the residue of SiO_2 and WO_3 weighed as suggested by Bertrand and Javillier, or the filtration might be performed in a Gooch crucible and the salt directly weighed after drying at 120° C. Both methods were tried.

Weighing as anhydrids.—The anhydrid method is obviously the quicker and easier of the two possibilities. A platinum crucible is naturally employed, from which the residue is easily removed. One drawback to the method results from the fact that when nicotin silicotungstate is decomposed by heat much carbon is deposited throughout the residual mass. If the ignition is performed carefully and with free access of air, such deposited carbon appears to be readily and completely burned away. The conditions are, however, evidently very favorable for the retention of unburned carbon or the reduction of WO_3 within the interior of the residue, which forms a coherent porous mass. Moreover, WO_3 is recognized to be appreciably volatile at a high temperature, hence protracted ignition of the residue must be avoided, though a high enough temperature must be reached to assure dehydration of silicic acid. Citation of an experiment performed will best serve to show the difficulties here involved. The precipitate obtained from about 0.06 gram of nicotin was filtered on paper, washed, and the wet paper and precipitate brought into a platinum crucible. After drying and carbonization over a Bunsen burner the crucible was inclined and carbon burned away at a gradually increased heat. After cooling and weighing, the crucible and contents were next subjected to successive periods of heating, and were weighed after each period. The results are shown in Table 2.

TABLE 2.—*Effect of continued ignition upon the residue of anhydrids from nicotin silicotungstate.*

Method of ignition.	Weight of crucible and residue.
	Grams.
After ignition over Bunsen burner	20.5029
After 5 additional minutes over Bunsen burner	20.5024
After 5 additional minutes over Teclu burner	20.5018
Do	20.5015
Do	20.5015
Do	20.5012
After 20 to 25 additional minutes over Teclu burner	20.5000
After 5 additional minutes over blast lamp	20.4997

NOTE.—The crucible itself lost 0.0004 gram during the experiment.

It is evident that the weight of the residue changes appreciably on ignition, the degree and rate—and even the direction—of the change depending naturally on such circumstances as the conditions of heating and the degree of porosity of the residue. The results of Table 2 indicate that heating over a Teclu burner for a period between five and ten minutes will give the most consistent results in practice. The residue forms a porous mass, usually a clear lemon-yellow on the outside but showing a decided greenish hue in the interior.

Weighing as salt.—The salt method is subject to three disadvantages when compared to the anhydrid method: (1) A Gooch crucible must be prepared and weighed for each determination; (2) the precipitate packs down tightly upon the felt under suction, so that filtration is slow; (3) the anhydrous salt is very hygroscopic—a fact not mentioned by Bertrand and Javillier—hence the crucible and contents, after drying, must be tightly inclosed in a weighing bottle during cooling and weighing. On the other hand, results obtained by the salt method are free from the special sources of error attached to the anhydrid method. The salt reaches constant weight at 120° C. and may be held at 130° C. without showing further loss.[1] Hence the method is of value as an ultimate reference method in cases where very exact and indubitable results are necessary. For ordinary purposes the anhydrid method appears sufficiently accurate and is certainly much more rapid and convenient.

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[1] The anhydrous salt theoretically contains 10.12 per cent nicotin. Before weighing the salt and Gooch crucible inclosed in the weighing bottle, the stopper of the latter must be loosened for an instant to permit equalization of air pressure.

ANALYTICAL RESULTS OBTAINED BY THE SILICOTUNGSTATE METHOD.

The silicotungstate method was first tested upon solutions of nicotin. The material bore the designation "highest purity" over the name of a firm of excellent reputation. That it was not absolutely pure was indicated by an evident opalescence when it was dissolved in dilute hydrochloric acid. A gram or two was weighed in a stoppered weighing bottle, washed into a measuring flask with slightly acidulated water, and made to volume. For the determination an aliquot was pipetted out, made to about 50 c. c., and sufficient dilute hydrochloric acid added to bring the amount present to between two-tenths and four-tenths of 1 per cent of concentrated hydrochloric acid. To the fluid so prepared was next added some excess of a silicotungstic acid solution, and the whole was well stirred and allowed to stand until the next day. By that time the precipitates, at first finely divided and settling very slowly, had become a mass of detached glittering crystals, settling quickly when agitated. The precipitate was filtered and washed with water containing 1 c. c. of concentrated hydrochloric acid in a liter, and then treated according to one or the other of the two methods outlined above. The results are given in the following table:

TABLE 3.—*Determination by silicotungstate method of nicotin in "Nicotin, highest purity."*

	Weight of nicotin taken.	Weight of anhydrous salt.	Weight of anhydrids.	Per cent nicotin.
	Gram.	*Gram.*	*Gram.*	
Solution A	0.05856		0.5103	99.34
	.05856		.5119	99.65
	.05856	0.5761		99.56
	.05856	.5756		99.47
	.05856	.5777		99.83
	.11712	1.1505		99.42
Solution B	.05506	.5405		99.34

The results of the foregoing table do not justly show the precise limits within which duplicate determinations upon a single nicotin solution may be made to check, for in the work no special precautions were taken outside of ordinary laboratory practice. In taking aliquots of the nicotin solution the ordinary 25 c. c. pipettes supplied to the laboratory were employed indifferently, as they happened to come to hand on different days. These pipettes, though supplied by reputable makers, were not calibrated, and a subsequent test of some of the stock in hand showed that the variation in volumes delivered was nearly of as great magnitude as the variation in the percentages of nicotin found in the samples.

In the above series of determinations the volume of filtrate and washings was not definitely noted, though it evidently averaged about

150 c. c. To determine whether any restriction of or correction for the volume of filtrate and washings might be necessary, an aliquot of Solution A containing 0.05856 gram nicotin was precipitated and filtered as before upon an S. & S. No. 589 filter paper, 9 cm. in diameter, and washed in the ordinary manner until the total volume of filtered liquid amounted to 500 c. c. The weight of residue obtained after ignition was 0.5116 gram, equivalent to 99.59 per cent nicotin. A repetition of the experiment, continuing washing until the total volume amounted to 900 c. c., gave 0.5120 gram residue, equivalent to 99.67 per cent nicotin. It is hence apparent that the precipitate is extremely insoluble, that it may be precipitated quantitatively from very dilute solution, and that no correction for volume of filtrate or washings is necessary.

THE DETERMINATION OF NICOTIN IN COMMERCIAL PREPARATIONS.

If the silicotungstate method is destined to receive recognition and wide employment as a reliable and useful method for the determination of nicotin, a matter of considerable practical importance will be a determination of the degree of closeness with which results thereby obtained may be expected to check with those of the Kissling method when both methods are applied to the same commercial nicotin preparations. Several such preparations, which had previously been carefully and repeatedly analyzed by the Kissling method, were examined. The results are contained in the following table. It must be noted that each figure given in the last two columns of the table under the "silicotungstate method" represents a single complete independent analysis. The results in these two columns, then, are comparable as complete duplicates, and not merely as duplicate determinations upon a single nicotin distillate.

TABLE 4.—*Comparative results obtained by Kissling and silicotungstate methods when applied to commercial nicotin preparations.*

Character of preparation.	Per cent nicotin.		
	Kissling method (mean result).	Silicotungstate method.	
		Weighed as salt.	Weighed as anhydrida.
Nicotin dip, No. 660	21.86	21.91	
Nicotin solution, No. 755	40.05		39.77 39.84
Tobacco extract, No. 768	8.36	8.32	8.35
Nicotin solution, No. 810	39.41		40.12 40.00
Tobacco extract, No. 842	2.94		2.92 2.93

In view of the distinctly different rationale of the two methods and the difference in the sources of possible error inherent in each, the above series of results show a rather surprising agreement. Apparently the results obtained by one method are about equally likely to be either higher or lower than the results obtained by the other.

THE EFFECT OF EXTRANEOUS BASES.

As already noted, ammonia and amins—but not pyridin—may be practically eliminated by the Kissling method if a sufficiently long period of extraction is employed. Up to the present time apparently the only attention paid to the possible presence of pyridin bases in nicotin solutions is contained in the work of Emery,[1] performed in the laboratories of this bureau some years ago. Nicotin is levogyratory, and Emery took advantage of this fact to work out a method for the polariscopic examination of nicotin distillates. Emery's method is not particularly accurate when considered as a method for the determination of nicotin, and the presence of pyridin bases can be inferred with assurance only if the amount of nicotin so found is notably lower than that obtained by the Kissling method.

Ammonia and amins, so far as known, are not precipitated by silicotungstic acid, nor are the anilins, as indicated by a test on a solution of anilin oil in dilute hydrochloric acid. Pure pyridin in slightly acid solution yielded a precipitate with silicotungstic acid at a dilution of 1 in 5,000 only when allowed to stand several hours; at a dilution of 1 in 10,000 no precipitate could be perceived after forty-eight hours. Pyridin bases from coal-tar creosote extracted in the laboratory proved to yield much less soluble precipitates, an immediate opalescence appearing upon the addition of silicotungstic acid to a solution of the bases containing, roughly, 1 part in 50,000, but no reaction could be observed at a dilution of 1 in 100,000. The precipitate formed from the higher pyridin bases was markedly different in appearance from nicotin silicotungstate, seeming to remain amorphous, and yielding a permanently cloudy fluid. It is therefore evident that the silicotungstate method can not be relied upon to eliminate more than small amounts of pyridin bases. It will, however, successfully detect such bases if present in quantity sufficient to introduce error in the determination of nicotin, for although the silicotungstates of some of the pyridin bases are difficulty soluble, they are much more soluble than nicotin silicotungstate; hence, if they are thrown on a filter and washed, the washings will long continue to yield a turbidity when tested with a dilute nicotin solution, exactly as would a very dilute solution of silicotungstic acid. To illustrate this point, 0.01 gram of the pyridin bases extracted from coal-tar creosote was completely precipitated from 50 c. c. of slightly acidulated solution with silicotungstic acid, and the precipitate brought on a filter and washed. When the volume of filtrate and washings amounted to over 500 c. c. fresh washings still yielded a faint but unmistakable opalescence when tested with a few drops of nicotin solution. The precipitate had then been reduced to a very small amount in the point of the filter and the experiment was discontinued.

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[1] Emery, James A. Estimation of nicotine in presence of pyridine. Journal of the n Chemical Society, vol. 26, No. 9, pp. 1113–1119, September, 1904.

Accordingly, it appears that the presence of pyridin bases in quantity sufficient to affect the accuracy of the determination of nicotin in commercial preparations will be betrayed by an apparent difficulty experienced in washing the precipitate free from excess of silicotungstic acid.

TESTS ON A RAPID MODIFICATION OF THE METHOD.

In ordinary work the natural system of routine will be to perform the steam distillation and precipitation on one day, leaving the precipitate to form and settle overnight. In exceptional cases, such as certain exigencies of factory control work, even so much delay may cause inconvenience. Hence an attempt was made to hasten the conversion of the precipitate into a filterable form.

A study was first made of the effect of various concentrations of hydrochloric acid and of various temperatures. The material employed was another sample of "pure nicotin" (sample C), evidently not so pure as those previously used. To about 1 gram of the material was added 5 c. c. of dilute hydrochloric acid (1 to 4) and the whole was made to 500 c. c. with distilled water. Portions of 50 c. c. were drawn with the same pipette for all determinations, and after dilution to 100 c. c. were acidified and precipitated with 10 c. c. of a 12 per cent solution of silicotungstic acid. The conditions of precipitation and the results obtained are given in the following table:

TABLE 5.—*Tests on rapid method of precipitation.*

No. of test.	Conditions of precipitation.	Weight of sample.	Weight of anhydrids.	Nicotin.
		Gram.	*Gram.*	*Per cent.*
1	Added 1 c. c. dilute HCl; stood 21 hours at 21° to 24° C. (recording thermometer); faintly cloudy when filtered	0.1034	0.8815	97.23
2	Added 2 c. c. dilute HCl; otherwise as above	.1034	.8824	97.33
3	Added 5 c. c. dilute HCl; otherwise as above	.1034	.8825	97.34
4	Added 10 c. c. dilute HCl; otherwise as above	.1034	.8834	97.44
5	Added 10 c. c. concentrated HCl; otherwise as above	.1034	.8802	97.09
6	Added 2 c. c. dilute HCl; stood about 18 hours at about 30° C.	.1047	.8943	97.34
7	Added 1 c. c. dilute HCl; stood 3 hours at 37.5° C., then in cold water for 15 minutes	.1034	.8809	97.17
8	Added 10 c. c. dilute HCl; otherwise as No. 7	.1034	.8809	97.17
9	Added 2 c. c. dilute HCl; on steam bath 20 minutes; in cold water 15 minutes	.1047	.8900	96.87
10	Added 2 c. c. dilute HCl; on steam bath 1 hour; in cold water 15 minutes	.1047	.8891	96.78
11	Added 1 c. c. dilute HCl; on steam bath 2 hours; in cold water 15 minutes	.1047	.8889	96.76
12	Added 2 c. c. dilute HCl; on steam bath ½ hour; at room temperature 1½ hours	.1034	.8776	96.80

Evidently precipitation may be successfully carried out in presence of considerable hydrochloric acid. In such case the liquid soon clears, but the crystals deposited are small and it is difficult to determine when the change to crystalline form is complete. Tests 2 and 3 made altogether much the best appearance and mark the limits of the amount of acid which should be added.

The obvious effect of heat in lowering the percentage of nicotin recovered was at first difficult to account for. Filtrates from such tests showed no further precipitation on long standing, and subsequent experiments in which the tests were allowed to stand over night before filtration showed similarly low results, namely, about 0.5 per cent lower than those obtained by precipitation at room temperature. The suspicion arose that the results obtained by precipitating hot were really the correct ones and that precipitates formed and settled at room temperature occluded silicotungstic acid. To settle this question portions of a nicotin solution were precipitated both hot and cold, the tests made cold of course being allowed to stand over night before filtration, while tests made hot were filtered after cooling for about one hour. The precipitates were filtered on paper, washed and dried. A portion of each precipitate was then placed in a weighed platinum crucible, dried to constant weight at 120° to 130° C., and the weight of the dried precipitate obtained. A weighing bottle was of course employed to hold crucible and precipitate during weighing. The crucibles and precipitates were next ignited, and the weights of the various residues were obtained. If the precipitate formed at room temperature occludes silicotungstic acid it should yield a residue greater than the theoretical and greater than the residue obtained from the precipitate formed in hot solution. That such was not the case is shown by the following results:

TABLE 6.—*Residues obtained by ignition of dried nicotin silicotungstate.*

Method of precipitation.	Weight of dried salt.	Weight of residue.	Residue on dried salt.
	Grams.	*Gram.*	*Per cent.*
Cold	1.008	0.8914	88.43
Do	.6902	.6105	88.45
Hot	.9848	.8724	88.59
Do	.6914	.6116	88.46

Theoretical per cent of residue—88.76.

The next set of experiments involved the formation and crystallization of the precipitate from a mechanically stirred liquid at room temperature, holding either nicotin or silicotungstic acid in excess throughout, so far as possible. Solution D of "pure nicotin" was employed. Results appear in Table 7.

TABLE 7.—*Results from precipitation of nicotin silicotungstate under various of "pure nicotin." Weight of sample taken for each test = 0.1019 gram.*

No. of test.	Conditions of precipitation.	Weight of anhydrids.	Nicotin.
		Gram.	*Per cent.*
1	Volume of solution, 100 c. c.; added 2 c. c. dilute HCl; left at room temperature about 20 hours	0.8662	96.91
2	Volume of solution and acid, as in test 1; heated on steam bath ½ hour, then left at room temperature overnight	.8613	96.37
3	Volume of solution and acid, as in tests 1 and 2; stirred mechanically overnight at room temperature	.8657	96.85
4	Volume of solution, 50 c. c.; added 1 c. c. dilute HCl; into this dropped from burette solution of 10 c. c. silicotungstic acid made to 50 c. c. and acidified with 1 c. c. dilute HCl, requiring 4 hours for complete addition of the reagent; liquid thoroughly stirred during addition of reagent and overnight; room temperature	.8593	96.13
5	Duplicate of test 4	.8595	96.16
6	Solution and reagent prepared as in tests 4 and 5, but precipitated under reverse conditions, i. e., by dropping the nicotin solution into the reagent; stirred overnight at room temperature	.8650	96.77

The results in Table 7 prove that the composition of the precipitate is variable. Either the precipitate formed in a cold solution in which silicotungstic acid is in excess contains too much silicotungstic acid or the precipitates formed in cold solutions in presence of excess of nicotin and in all hot solutions contain too much nicotin. The results shown in Table 6 render the first hypothesis unlikely. They do, however, permit the second hypothesis to be entertained, for if too much nicotin were in any case contained in the precipitate it is possible that excess nicotin might be volatilized during the process of drying at 120° to 130° C. and hence leave the dried precipitate of normal composition. Altogether the results given in Tables 6 and 7 indicate that quantitative results can be obtained only when nicotin is precipitated at not over 30° C. by an immediate excess of silicotungstic acid. The crystals of silicotungstate thus obtained appear to possess a composition which is uniform and practically that demanded by theory.

In performing the experiments noted in Table 7 it was observed that freshly precipitated nicotin silicotungstate very quickly became crystalline under the action of a mechanical stirrer. That thorough and continued agitation is all that is necessary to secure a simple and accurate rapid modification of the method is apparent from the following results:

TABLE 8.—*Tests on rapid method based on mechanical stirring, using solution D of "pure nicotin." Weight of sample taken for each test = 0.1019 gram.*

No. of test.	Conditions of precipitation.	Weight of anhydrids.	Nicotin.
		Gram.	*Per cent.*
1	Volume of solution, 100 c. c.; added 2 c. c. dil. HCl and 10 c. c. silicotungstic acid; stirred over night. (Taken from Table 7, test 3, for comparison.)	0.8657	96.85
2	As test 1, except that stirred ½ hour only and filtered at once	.8645	96.72
3	As test 2, except that added 5 c. c. dil. HCl	.8638	96.64

In both test 2 and test 3 a change in the character of the precipitate had become apparent in the first 10 minutes; at the end of the second 10 minutes the change to a crystalline form appeared to be complete.

THE STEAM DISTILLATION OF NICOTIN PREPARATIONS.

Since nicotin is evolved from boiling dilute solutions with extreme slowness, in the steam distillation of nicotin preparations it is always desirable to keep the volume of liquid in the distillation flask at the lowest practicable amount. This is essential in the execution of the Kissling process, where the distillate is later to be titrated, and is there easily accomplished, since the liquid in the flask contains but a comparatively small amount of salts and other extraneous matter. Many commercial preparations contain a large amount of such extraneous matter, and during direct distillation consequently demand that a comparatively large volume of fluid be maintained in the distillation flask, with the result that a large volume of distillate must be collected in order to capture the last traces of nicotin. In general, this constitutes no serious drawback to the application of the silicotungstate method, for the extreme insolubility of nicotin silicotungstate admits of its quantitative separation from very dilute solutions of nicotin. The greater length of time required in such a direct steam distillation of a nicotin preparation is largely compensated in practice by the fact that when once properly started the distillation will progress without the close personal attention on the part of the analyst which is required during the steam distillation of the Kissling process, where the maintenance of the volume of liquid at its lowest possible amount is of great importance. In the case of high percentage nicotin solutions it is most convenient to count on a final volume of 1,000 c. c. for the distillate, in which case, if between 1 and 2 grams of nicotin are present in the whole, a 50 or 100 c. c. aliquot will be a proper and convenient amount to employ for precipitation. In the case of low percentage preparations the distillate may amount to 1,500 c. c. or more, and it is most conveniently handled by transferring to a 2,000 c. c. graduated stoppered cylinder and making to the next 100 c. c. mark.

In handling such low percentage preparations it is, of course, entirely possible, following the method of Bertrand and Javillier, first to precipitate the nicotin with silicotungstic acid, and then to subject the collected precipitate, after adding sufficient alkali, to distillation with steam, thus securing a small volume of liquid in the distillation flask and a consequent small volume of distillate. A serious practical objection to this method is the considerable necessary consumption of the expensive silicotungstic acid. It is possible that some other less costly material may be appropriate for such a preliminary precipitation, and in fact some preliminary experiments on the use of picric acid have been tried in this laboratory. But if it can possibly be avoided it seems very undesirable to introduce such a complication into a method which is otherwise consistent and uniform. At any rate, the proper working out and testing of such a method would require considerable time. For the present, if it is necessary to handle any nicotin preparation which absolutely will not permit distillation with steam as the first step, it is always possible to extract the material with ether after the Kissling method—restricting the time of extraction to that necessary simply to extract all nicotin—and to proceed with the residue in the extraction flask according to the regular silicotungstate method.

SUMMARY AND CODIFIED DIRECTIONS FOR ROUTINE WORK.

It appears, then, that the silicotungstate method, a logical outgrowth from the work of Bertrand and Javillier, is placed upon a firm basis as a scientifically sound and technically practical method for the accurate and convenient determination of nicotin in nicotin solutions and tobacco extracts. The results obtainable by the method theoretically should be more accurate than the results afforded by the Kissling method, a hypothesis supported by the results of the experimental work herein cited. Each step in the process and the completeness of each operation may be tested as the work proceeds, and but little is left dependent upon the judgment of the operator. The method is likewise indicated to excel in convenience and economy, for though it employs an expensive reagent it demands but comparatively small quantities, and although it is a gravimetric method, it is simple and rapid, yielding definite results susceptible of close duplication with no excessive demands upon the time or skill of the analyst.

From the data of experimental work the following codified directions may be offered for the routine execution of analyses by the silicotungstate method:

Weigh out such an amount of the preparation as will contain preferably between 1 and 2 grams of nicotin, except in the case of extracts high in extraneous matter, where not in excess of 30 grams should be employed; wash with water into a 500 c. c. round-bottomed flask; add 1 to 1½ grams paraffin, a few small pieces of pumice, and caustic soda solution to strong alkalinity (5 to 10 c. c. of NaOH solution, 1 to 2). Distill in a rapid current of steam through a three-bend connecting tube, a condenser, and adapter into 10 c. c. of dilute hydrochloric acid (1 to 4) in a capacious flask. When distillation is well started, apply heat to the distillation flask (a Babo funnel is most convenient) to reduce the volume of liquid as far as practicable without bumping or undue separation of insoluble matter. Continue distillation until a few cubic centimeters of the distillate collected from the condenser after removal of the adapter show no cloud or opalescence when treated with a drop of silicotungstic acid solution followed by a drop of dilute hydrochloric acid (1 to 4). Prove alkalinity of the residue in the distillation flask with phenolphthalein solution or paper.

Make the distillate to convenient volume, mix well and pass through a large dry filter, discarding a liberal first portion of the filtrate, and test a portion with methyl orange to assure its acidity. Pipette into a beaker an aliquot containing about 0.1 gram nicotin, add for each 100 c. c. of liquid 3 c. c. of dilute hydrochloric acid (1 to 4)—or more, if indicated necessary by the test with methyl orange—and add 1 c. c. of a 12 per cent solution of silicotungstic acid for each 0.01 gram nicotin supposed to be present.[1] Stir thoroughly and let stand for eighteen hours.[2] Then stir up the precipitate, making sure that it settles quickly and completely in crystalline form; filter on a quantitative paper, and wash with cold water containing 1 c. c. of concentrated hydrochloric acid per liter. Test the first portion of the filtrate with a few drops of nicotin distillate to prove excess of silicotungstic acid.

Continue washing for two or three fillings of the filter after no more opalescence appears when a few cubic centimeters of fresh filtrate is tested with a few drops of nicotin distillate. Bring the wet paper and precipitate into a weighed platinum crucible, using a scrap of moistened filter paper to transfer any precipitate which may have crept up the sides of the funnel; dry carefully, carbonize, and finally burn off the carbon at as low a temperature as possible. Gradually increase the heat and occasionally rotate the crucible to expose all parts of the residue. At last ignite the inclined crucible thoroughly over the full heat of a Bunsen burner, finishing with five to ten minutes—not longer—over a powerful Teclu burner, or five minutes over a moderately powerful blast lamp. Cool in a desiccator. The weight of the residue multiplied by 0.114[3] affords the weight of nicotin in the aliquot taken for precipitation. If the highest possible degree of accuracy is desired, filter the precipitate on a weighed Gooch crucible, dry at 125° C., and weigh the anhydrous nicotin silicotungstate as indicated on page 13.

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[1] An excess of 17 to 18 per cent over the amount theoretically necessary is here provided, allowing for all ordinary variations.

[2] This length of time is probably more than is necessary, but in the absence of positive evidence it is wiser to prescribe it. For quick results use a mechanical stirrer, as indicated on page 19.

[3] The factor given by Bertrand and Javillier is 0.1139 and is apparently based upon an erroneous calculation, since other calculations cited by them check with those performed in this laboratory upon the basis of the most recent available atomic weights. The factor is actually slightly over 0.1140.

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THE ESTIMATION OF TOTAL SOLIDS IN MILK BY THE USE OF FORMULAS.

BY

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S. C. Thompson, *in charge of Dairy Manufacturing Investigations.*
L. A. Rogers, *in charge of Research Laboratories.*
George M. Whitaker, *in charge of Market Milk Investigations.*
Robert McAdam, *in charge of Renovated Butter Inspection.*

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., November 26, 1910.

Sir: I have the honor to transmit herewith, and to recommend for publication in the bulletin series of this bureau, a manuscript entitled "The Estimation of Total Solids in Milk by the Use of Formulas," by R. H. Shaw, of the Dairy Division of this bureau, and C. H. Eckles, of the Missouri Agricultural Experiment Station. The experimental work herein described forms a part of the investigations concerning milk which are being conducted at the Missouri station in cooperation with this bureau.

Owing to the necessity for some more rapid method of calculating the solids in milk than the usual laboratory procedure, the estimation of these constituents by means of formulas has been a common dairy practice for some years; and while a certain amount of error was known to exist in such calculations, it was assumed to be small enough to be negligible for most practical purposes. Inasmuch, however, as a number of formulas are in use, each differing slightly in results from the others, it became a question of some importance to determine which of them was the most accurate.

With the object of solving this problem the authors have made searching tests under exacting conditions of several of the best known formulas, and have in addition devised an improved lactometer which, with a table based upon the results of the work described in this bulletin, is believed to furnish a method which is more nearly accurate than any at present in use.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.

Introduction........ 5

Synopsis of formulas in vogue........ 6

Experiments to compare the accuracy of existing formulas........ 7

  Methods of calculation and terminology........ 8

  Method of sampling........ 8

  Method of determining specific gravity and total solids........ 9

Comparison of the formulas with gravimetrically determined results........ 9

The specific gravity of milk solids........ 11

Experiments to determine accuracy of lactometers........ 15

Tests of Babcock formula and new lactometer with individual milkings........ 17

Effect of temperature on specific gravity of milk........ 19

  Recknagel's phenomenon........ 20

How to use the modified lactometer and table........ 21

  Method........ 21

  Directions for using the table........ 22

Summary and conclusions........ 25

Appendix........ 26

ILLUSTRATION.

Page.

Fig. 1. Lactometer designed for use in experimental work........ 16

THE ESTIMATION OF TOTAL SOLIDS IN MILK BY THE USE OF FORMULAS.

INTRODUCTION.

Various formulas have been in use for a number of years as a means of determining the total solids in milk when the specific gravity and percentage of fat are known. This rapid estimation of the total solids is a useful and convenient method for purposes where exactness is not required. Among the more common uses that have been made of this method is the preliminary examination of market milk by inspectors and the detection of adulterations at cheese factories.

Recently certain organizations representing the dairy breeds of cattle have considered the advisability of reporting the percentage of total solids as well as of fat in making official tests of individual cows. It therefore became a question as to whether the determination of the total solids by means of the formulas and the instruments in common use for finding the specific gravity was feasible and accurate enough.

In view of this question Mr. Ed. H. Webster, then Chief of the Dairy Division of the Bureau of Animal Industry, requested the authors to take up the problem with the view of testing the accuracy of the estimation of total solids by the several formulas in common use and to suggest improvements looking toward greater accuracy in finding the specific gravity without making the determination impracticable for use by such men as usually have charge of official testing.

For the present purpose milk may be regarded as composed of fat and milk plasma, the latter being made up of water and the various milk solids not fat, such as the proteins, sugar, ash, and other solids. Fat, having a specific gravity less than water, has the effect of lowering the specific gravity of milk, while the plasma solids, having a specific gravity greater than water, have the effect of raising it. It is clear, then, that a relation exists between the specific gravity of milk and its percentage of fat and solids not fat. The various formulas for calculating total solids or solids not fat, when the other two factors are given, are based upon this relation.

It is not the purpose of this bulletin to bring out a new formula or to suggest modifications or revisions of those already in use. The main objects of the investigation herein reported were: (1) To compare the percentages of total solids calculated by means of certain formulas in general use with those obtained gravimetrically in the laboratory; (2) to test under more exacting conditions the formula which yields results closest to gravimetrically determined total solids, and (3) to devise a new or modify an existing lactometer with which the specific gravity may be more accurately determined.

The authors desire to acknowledge their indebtedness to A. E. Perkins and G. C. Payne, of the Dairy Division and Missouri Agricultural Experiment Station, for assistance rendered in obtaining the data included in this bulletin.

SYNOPSIS OF FORMULAS IN VOGUE.

Behrend and Morgen[1] published in 1879 the first formula of which there is any record which attempts the calculation of total solids from the specific gravity and the percentage of fat. They were closely followed in the same year by Clausnitzer and A. Mayer,[2] who published another formula. These two formulas were, however, based on inaccurate data and have since been abandoned. Since that time numerous other formulas have been proposed, among them being one by Fleischmann and Morgen.[3] In this formula the specific gravity of butter fat was assumed to be 0.94. This was changed to 0.93 by Fleischmann,[4] and the formula thus revised is still in general use and is one of those compared in this investigation. Hehner's[5] formula appeared in 1882, that of Halenke and Moeslinger[6] in 1886, and that of Hehner and Richmond[7] in 1888. The latter formula was revised in 1894 by Richmond,[8] and the revised form is known as Richmond's new formula. Babcock[9] published his formula in 1891, but changed it four years later.[10]

Comparisons of the various formulas with gravimetrically determined total solids have appeared from time to time. Such comparisons were made in 1889 by Woll,[11] who worked with the Fleischmann and the Hehner and Richmond formulas. In his conclusions, which are in favor of the Fleischmann formula, he states that it may be used to advantage for calculation of total solids if the specific gravity of milk is taken at 15° C.

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[1] Journal für Landwirtschaft, Band 27, p. 249. Berlin, 1879.

[2] Forschungen auf dem Gebiete der Vieh-haltung und ihrer Erzeugnisse, p. 265. Bremen, 1879.

[3] Journal für Landwirtschaft, Band 30, p. 293. Berlin, 1882.

[4] Journal für Landwirtschaft, Band 33, p. 251. Berlin, 1885.

[5] Analyst, Vol. VII, p. 129. London, 1882.

[6] Chemiker-Zeitung, Jahrg. 10, semester 1, Chemisches Repertorium, p. 8. Cöthen, 1886.

[7] Analyst, vol. 13, p. 26. London, 1888.

[8] Proceedings of the Eleventh Annual Convention of the Association of Official Agricultural Chemists, Washington, D. C., Aug. 23-25, 1894, United States Department of Agriculture, Bureau of Chemistry, Bulletin 43, p. 181.

[9] Eighth Annual Report of Wisconsin Agricultural Experiment Station, 1891, p. 292. Madison, 1892.

[10] Twelfth Annual Report of Wisconsin Agricultural Experiment Station, 1895, p. 120. Madison, 1896.

[11] Agricultural Science, vol. 3, p. 129. State College, Pa., 1889.

In the early nineties the Association of Official Agricultural Chemists made some comparisons of the Fleischmann, the Hehner and Richmond, the Babcock (original), and the Richmond formulas. Their results are published in the proceedings of their tenth [1] and eleventh [2] annual conventions, and in commenting on the same they state that the Hehner and Richmond formula gave figures which compared best with those obtained gravimetrically.

EXPERIMENTS TO COMPARE THE ACCURACY OF EXISTING FORMULAS.

In a cooperative experiment between the Missouri Agricultural Experiment Station and the Dairy Division of the Bureau of Animal Industry, United States Department of Agriculture, a study was made of the changes in chemical composition which milk undergoes during the natural period of lactation. Among many other factors the specific gravity and the percentages of fat and total solids were determined under controlled conditions. These determinations were made on 12 animals through one entire lactation period, and on 2 of the animals through two entire lactation periods. Having these data at hand, it became purely a matter of substitution to apply the figures obtained for the specific gravities and the percentages of fat in some of the most frequently used formulas for determining total solids when these two factors are known, and comparing the figures so obtained with the corresponding percentages of total solids determined gravimetrically. As stated in the introduction, several formulas have been published, but perhaps of these the four most used are those derived by Babcock (revised), Hehner and Richmond,[3] Richmond, and Fleischmann. In the general lactation experiment above referred to the samples were taken from the very beginning of the lactation period to the very end of the period, but since the purpose of this investigation is to show the application of various formulas in determining total solids in normal milk, it was thought best to exclude the extremes from the comparisons, and so the figures, except when otherwise stated, refer to milk of normal composition.

The 12 animals used in the investigation included 3 each of 4 breeds—Holstein-Friesian, Jersey, Ayrshire, and Shorthorn. According to the general plan these animals were kept on a uniform ration throughout the entire milking period in order to eliminate possible changes in the composition of the milk due to feed. This ration consisted of alfalfa hay, three-fifths, the other two-fifths being made up of corn 8 parts, bran 1 part, and oats 1 part. The ratio between the hay and the grain was kept the same at all times. The cows were kept in the barn during the night and in an adjoining lot having no grass or other food during the day. The animals were fed and milked twice daily, at 5 a. m. and 4 p. m. The ration served to keep the animals in good condition, and the production of milk was about typical of the breeds, although not equal to that produced previously by the same animals when opportunity was given to vary the ration and adapt it to the needs of the individual.

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[1] Proceedings of the Tenth Annual Convention of the Association of Official Agricultural Chemists, Chicago, Aug. 24-26, 1893. U. S. Department of Agriculture, Bureau of Chemistry, Bulletin 38, p. 107.

[2] Proceedings of the Eleventh Annual Convention of the Association of Official Agricultural Chemists, Washington, D. C., Aug. 23-25, 1894. U. S. Department of Agriculture, Bureau of Chemistry, Bulletin 48, p. 182.

[3] The formula of Hehner and Richmond was compared in the same way as the others, but the results were so nearly identical with those of the Babcock formula that it was thought best to omit them from this bulletin.

METHODS OF CALCULATION AND TERMINOLOGY.

In preparing the mass of calculations involved in this bulletin free use was made of tables prepared by the several authors of the formulas. In calculating and averaging percentages the rule followed was to discard the third decimal figure when it was less than 5, and to increase the second by one when it was 5 or more. This will explain what may appear to be discrepancies in some of the tables.

To be strictly accurate the average of a series of specific-gravity determinations must be made by first converting the result for each determination into terms of specific volume. These figures may then be averaged in the usual manner and the resulting average converted back into terms of specific gravity. The error introduced, however, by simply dividing the sum of the specific gravities by the number of determinations was so very small that the averages given in this bulletin were all made in this way.

In order to avoid confusion, the term "plasma" is employed to designate whole milk minus the fat; "plasma solids" to designate the solids in milk minus the fat; and "total solids" the solids including the fat.

METHOD OF SAMPLING.

The milk was weighed after milking and mixed by pouring it back and forth from one pail into another. A sample of about 1 quart was placed in a glass jar bearing the number of the cow and the number of pounds for that particular milking, and delivered to the laboratory. A certain number of cubic centimeters per pound were then measured out and placed in a covered receptacle to make up a composite sample to represent a week's milk from that particular cow. Formaldehyde was added in the proportion of 1 part to 5,000 to preserve the sample. At the end of the week the composite sample was thoroughly mixed and a subsample taken for chemical analysis.

METHOD OF DETERMINING SPECIFIC GRAVITY AND TOTAL SOLIDS.

The specific gravity of the milk was determined at 15° C. by means of a Westphal balance.

The determinations of fat and total solids were made by the Babcock asbestos method. A woolly asbestos was used in perforated copper cylinders, and the determinations were conducted according to the official method as described in Bulletin 107 (revised) of the Bureau of Chemistry, United States Department of Agriculture.

COMPARISON OF THE FORMULAS WITH GRAVIMETRICALLY DETERMINED RESULTS.[1]

The main table showing the comparisons of the three formulas in the individual cases is found in the appendix. Tables 1, 2, and 3, immediately following, are made up of averages from figures in the main table. No explanation will be needed to show how the various figures are obtained. A study of the tables will show that in the case of every cow, regardless of breed or individuality, the Babcock formula yielded results closest to those obtained by gravimetric determination of the total solids. A comparison of the results obtained by the Babcock formula with the gravimetric results shows that 256, or 59.53 per cent, of the 430 cases agree within 0.25 per cent, and that 389, or 90.46 per cent, agree within 0.5 per cent. Using Richmond's formula in the same way, 360, or 83.7 per cent, of the cases fall within the prescribed limit of 0.5 per cent. Likewise Fleischmann's formula shows 309 cases of agreement, or 71.85 per cent. With the Hehner and Richmond formula, the figures of which are omitted from this publication for reasons previously stated, there was a similar agreement in 387, or 89.99 per cent, of the cases, showing that this formula yields results practically identical with those derived from the Babcock formula.

A study of Table 4 will reveal the fact that the calculated figures from the Babcock formula do not differ from the gravimetric figures in any uniform direction, but that the plus and minus differences nearly counterbalance. That they do nearly counterbalance is shown conclusively in Table 3, where it will be seen that the average figure for the calculated solids for the entire series of comparisons differs only 0.07 per cent from the corresponding average figure determined gravimetrically.

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[1] In some cases in this bulletin the specific gravities are given in terms of Quevenne degrees. These degrees, of course, refer to the arrangement of the scale on the style of the lactometer known as the Quevenne lactometer. Quevenne degrees are converted into specific gravity by dividing by 1,000 and then adding 1 to the quotient. For example, if the Quevenne reading is 32.5 the specific gravity is 1.0325.

TABLE 1.—*Number of instances where the total solids calculated by the Babcock, Richmond, and Fleischmann formulas lie within stated limits of the gravimetrically determined total solids.*

[Number of cases.]

Cow No.	Between 0 and 0.24 per cent.			Between 0.25 and 0.49 per cent.			Between 0.50 and 0.74 per cent.			Between 0.75 and 1 per cent.			Over 1 per cent.		
	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.
4	20	15	10	4	6	9	1	4	4	....	....	2	....	....	....
99	15	14	10	16	9	7	6	12	11	....	2	9	....	....	....
118	15	18	14	16	11	11	2	4	8	....	....	....	....	....	....
Total for breed	50	47	34	36	26	27	9	20	23	....	2	11	....	....	....
205	30	26	16	12	13	17	3	6	10	1	1	2	1	1	2
206	20	17	16	15	15	12	5	7	7	....	1	5	....	....	....
209	30	26	23	6	10	13	9	6	2	1	3	7	....	1	1
Total for breed	80	69	55	33	38	42	17	19	19	2	5	14	1	2	3
300	12	15	15	11	9	5	3	1	5	....	1	1	....	....	....
300	14	14	8	9	7	9	1	3	6	....	....	1	....	....	....
301	17	14	11	5	9	8	3	2	4	....	....	2	....	....	....
301	23	22	16	12	11	12	1	3	7	1	....	1	....	1	1
302	9	8	4	2	2	6	1	1	1	....	1	1	....	....	....
Total for breed	75	73	54	39	38	40	9	10	23	1	2	6	....	1	1
400	23	21	14	11	10	14	1	3	5	....	1	2	....	....	....
402	21	17	11	7	10	10	1	2	7	....	....	1	....	....	....
403	7	3	3	7	8	5	....	3	6	....	....	....	....	....	....
Total for breed	51	41	28	25	28	29	2	8	18	....	1	3	....	....	....
Total cases	256	230	171	133	130	138	37	57	83	3	10	34	1	3	4

TABLE 2.—*Data of Table 1 expressed in percentages.*

[Per cent of cases.]

Cow No.	Between 0 and 0.24 per cent.			Between 0.25 and 0.49 per cent.			Between 0.50 and 0.74 per cent.			Between 0.75 and 1.00 per cent.			Over 1 per cent.		
	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.	Babcock.	Richmond.	Fleischmann.
4	80	60	40	16	24	36	4	16	16	....	....	8	....	....	....
99	40.54	37.84	27.03	43.24	24.32	18.92	16.22	32.43	29.73	....	5.41	24.32	....	....	....
118	45.45	54.55	42.42	48.49	33.34	33.34	6.06	12.12	24.24	....	....	....	....	....	....
Per cent for breed	52.64	49.48	35.80	37.89	27.37	28.41	9.47	21.05	24.21	....	2.10	11.58	....	....	....
205	63.83	55.33	34.04	25.54	27.66	36.16	6.38	12.76	21.27	2.13	2.13	4.26	2.13	2.13	4.26
206	50	42.50	40	37.50	37.50	30	12.50	17.50	17.50	....	2.50	12.50	....	....	....
209	65.21	56.52	50	13.04	21.74	28.26	19.56	13.05	4.35	2.17	6.52	15.22	....	2.17	2.17
Per cent for breed	60.15	51.87	41.35	24.80	28.57	31.51	12.77	14.29	14.29	1.50	3.67	10.52	.75	1.50	2.26
300	46.15	57.69	57.69	42.32	34.61	19.23	11.53	3.85	19.23	....	3.85	3.85	....	....	....
300	58.33	58.34	33.33	37.50	29.17	37.50	4.17	12.50	25	....	....	4.17	....	....	....
301	68	56	44	20	36	32	12	8	16	....	....	8	....	....	....
301	62.16	59.46	43.24	32.44	29.73	32.44	2.70	8.11	18.92	2.70	....	2.70	....	2.70	2.70
302	75	66.68	33.34	16.66	16.66	50	8.34	8.33	8.33	....	8.33	8.33	....	....	....
Per cent for breed	60.49	59.68	43.55	31.46	30.65	32.26	7.26	8.07	18.55	.81	.81	4.84	....	.81	.81
400	65.70	60	40	31.44	28.57	40	2.86	8.57	14.29	....	2.86	5.71	....	....	....
402	72.41	58.62	37.93	24.14	34.48	34.48	3.45	6.90	24.14	....	....	3.45	....	....	....
403	50	21.43	21.43	50	57.14	35.72	....	21.43	42.85	....	....	....	....	....	....
Per cent for breed	65.39	51.29	34.61	32.05	37.18	37.18	2.56	10.26	24.36	....	1.28	3.85	....	....	....
Total per cent	59.53	53.48	39.76	30.93	30.23	32.09	8.60	13.25	19.30	.70	2.32	7.90	.23	.70	.93

TABLE 3.—*Average specific gravity, nitrogen, sugar, fat, and total solids for each cow, each breed, and the total average.*

Cow No.	Specific gravity of milk.	Total nitrogen.	Sugar.	Fat.	Solids not fat.	Total solids—gravimetric.	Total solids—Babcock.	Total solids—Richmond.	Total solids—Fleischmann.
	Degrees.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
4	33.4	0.61	4.79	4.88	9.23	14.19	14.21	14.35	14.47
99	32.9	.53	4.99	4.70	8.95	13.65	13.87	14.02	14.13
118	34.2	.65	4.87	5.39	9.66	15.05	15.02	15.16	15.28
Average for breed	33.5	.60	4.88	4.99	9.28	14.30	14.37	14.51	14.63
205	32.9	.47	5.09	3.23	8.78	11.99	12.10	12.24	12.37
206	29.8	.44	4.25	2.96	7.98	10.94	11	11.16	11.26
209	31.4	.52	4.29	3.09	8.45	11.51	11.54	11.70	11.80
Average for breed	31.4	.48	4.54	3.09	8.40	11.48	11.55	11.70	11.81
300	33.3	.60	4.86	4.20	9.23	13.43	13.38	13.52	13.64
300	31.8	.48	4.84	3.49	8.56	12.05	12.12	12.28	12.39
301	33.1	.54	5.02	4.28	9.11	13.39	13.41	13.54	13.67
301	32.6	.51	5	3.84	8.85	12.72	12.77	12.93	13.04
302	31.7	.55	4.78	4.49	8.77	13.24	13.32	13.48	13.58
Average for breed	32.5	.54	4.90	4.06	8.90	12.96	13	13.15	13.26
400	33.9	.53	5.09	3.86	9.22	13.08	13.12	13.24	13.38
402	33.8	.54	4.97	4.04	9.16	13.20	13.28	13.41	13.55
403	33.2	.49	5.23	3.34	8.79	12.12	12.31	12.49	12.50
Average for breed	33.6	.52	5.10	3.75	9.06	12.80	12.90	13.05	13.14
Total average	32.8	.54	4.86	3.97	8.91	12.89	12.96	13.10	13.21

THE SPECIFIC GRAVITY OF MILK SOLIDS.

Assuming that milk is a mixture of milk plasma and fat, it will be seen at once that if the specific gravities of the fat and of the plasma solids were constant quantities the relation of the specific gravity of the milk, the percentage of fat, and the percentage of plasma solids could be expressed mathematically. From such a mathematical relation it would be but a step to derive a formula for finding any one of these factors when the other two were given.

The specific gravity of butter fat is about 0.93 at 15° C., the variation from this figure in different samples being so slight as to be negligible for all practical purposes. It may then be considered as a constant, and is so treated in the formulas compared in the previous part of this bulletin.

The specific gravity of the plasma solids is not a constant, but varies in different samples of milk. This variation is, however, not a large one, being generally within comparatively narrow limits in normal milk. It is because of these narrow limits that formulas are admissible.

Richmond,[1] working in England, found from the analyses of over 200 samples of milk the average specific gravity of the plasma solids to be 1.616. Fleischmann [2] obtained the figure 1.6007 from the average of a large number of samples from cows in North Germany. The latter investigator has published a formula for calculating the specific gravity of the plasma solids when the specific gravity of the milk, the percentage of fat, and the percentage of total solids are known, thus:

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[1] Richmond's Dairy Chemistry, p. 6. London, 1899.

[2] Fleischmann's Book of the Dairy, p. 33. London, 1896.

$$n=\frac{s\times o\,(t-f)}{100\times o-s\times o\,(100-t)-sf}$$

The values for the specific gravity of plasma solids, the specific gravity of milk, the percentage of total solids, the percentage of fat, and the specific gravity of the fat in this formula are denoted, respectively, by the letters *n*, *s*, *t*, *f*, and *o*.

Applying this formula to the average of the 430 determinations given in Tables 1, 2, and 3 it is found that 1.638 is the average specific gravity of the plasma solids.

It has already been noted that the Fleischmann formula gave figures higher than the Richmond, which in turn gave figures higher than the Babcock. Since the average of the total solids determined by the latter agreed very closely with our gravimetrically determined total solids, it may be inferred that if Babcock had given a figure for the specific gravity of plasma solids to correspond with his revised formula, it would have been very close to our figure. It may be seen that the difference between these three formulas is largely due to the difference in the specific gravity of the plasma solids of the milk chosen by the respective investigators to represent the normal.

As previously noted, Babcock's original formula appeared in 1891 and its corrected form in 1895. As it originally stood it was:

$$\text{Plasma solids}=\left(\frac{100\,S-Sf}{100-1.0753\,Sf}-1\right)\times(100-f)\,2.6$$

In the above, *S* represents the specific gravity and *f* the percentage of fat. Subsequent to its publication Babcock found the constant, 2.6, too high and changed it to 2.5, so that the formula as it now stands is:

$$\text{Plasma solids}=\left(\frac{100\,S-Sf}{100-1.0753\,Sf}-1\right)\times(100-f)\,2.5$$

This revised form is the one used in the former part of this bulletin.[1] In deriving this formula Babcock assumes that the difference between the specific gravity of water and that of milk plasma is nearly in direct proportion to the solids which the plasma contains,[2] and that if this difference be divided by a constant factor which represents the increase in specific gravity caused by 1 per cent of plasma solids the result will be the percentage of solids in the plasma. If the percentage of solids in the plasma found in this way be multiplied by the percentage of plasma in the milk and the product divided by 100, the result will be the percentage of plasma solids in the milk Let

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[1] With the exception of the introduction of a few intermediate steps and the substitution of the term plasma for serum, the subject-matter showing the derivation of the Babcock formula was taken almost verbatim from his original article, to which reference has already been made. When the revised formula was published no figures for the values of *x* and *a* were given. Rather than use his original figures, which are now obsolete, it was thought advisable to use our own figures for the purposes of illustration; hence the factor 2.47 will be found in the resulting formula instead of 2.5.

[2] Dr. Babcock calls attention to the fact that this assumption is not quite correct (see Twelfth Annual Report, Wisconsin Agricultural Experiment Station, p. 121), since if the plasma solids were always of the same composition the specific gravity of the plasma solids and the plasma would change at different rates. This error, he states, is a very small one and is counterbalanced by the variation in the composition of the plasma solids in normal milk.

f = percentage of fat in any milk.
$100-f$ = percentage of plasma in any milk.
S = specific gravity of milk at 60° F.
0.93 = specific gravity of butterfat at 60° F.
x = specific gravity of plasma at 60° F.
a = increase in the specific gravity of the plasma caused by 1 per cent of plasma solids.

Then, I.—

Percentage of plasma solids in any milk $=\frac{x-1}{a}\times\frac{100-f}{100}$

$\frac{100}{S}$ = volume in c. c. of 100 grams of milk.

$\frac{100-f}{x}$ = volume in c. c. of plasma in 100 grams milk.

$\frac{f}{0.93}$ or $1.0753f$ = volume in c. c. of fat in 100 grams milk.

Since the volume of the milk equals the sum of the volumes of fat and plasma, then

$$\frac{100}{S}=\frac{100-f}{x}+1.0753f$$

Clearing of fractions and reducing

$$100x=100S-Sf+1.0753Sfx$$

Transposing and combining

$$x(100-1.0753Sf)=100Sf-Sf$$

$$\text{Or } x=\frac{100S-Sf}{100-1.0753Sf}$$

II. By first getting a value for x from a large number of analyses a is found. Subtract 1 from x and divide the remainder by the percentage of solids which the plasma contains. The percentage of solids in the plasma is found by dividing the percentage of plasma solids in the milk by the percentage of plasma $(100-f)$ and multiplying by 100.

The value of a in our work is 0.004044.

Substituting the value of x and a in I.

$$\frac{\frac{100S-Sf}{100-1.0753Sf}-1}{.004044}\times\frac{100-f}{100}$$

then

$\left(\frac{100S-Sf}{100-1.0753Sf}-1\right)\times(100-f)\times 2.4703$ = percentage of plasma solids in the milk.

The percentage of total solids is found by adding the percentage of fat to the percentage of plasma solids.

It will readily be seen that Babcock's value of representing the increase in specific gravity of the plasma caused by 1 per cent of the plasma solids depends directly upon the specific gravity of the plasma solids. This is a common point of weakness in all formulas derived for the same purpose.

Since the plasma solids are composed of several solids, chief of which are milk sugar, proteins, and ash, the specific gravity of the plasma solids must depend upon the specific gravity of the various components taken individually. Richmond [1] states that the specific gravity of milk sugar is 1.666, that of the proteins 1.346, and that of the ash 4.12. A change in the ratio of the milk sugar and the proteins will affect the specific gravity of the plasma solids and consequently the value of *a*. With a milk containing an abnormally high percentage of sugar the total solids calculated by the formulas would be theoretically too high. They would be too low under the reverse condition.

This is very well shown in the table below, the results in which are obtained from milk of a cow of the Shorthorn breed at the parturition period. As is well known, the milk taken at this time is abnormally high in proteins, while the milk sugar is abnormally low. The cow freshened on the morning of October 23.

TABLE 4.—*Showing application of the Babcock formula to colostrum milk.*

Date.	Total nitrogen.	Fat.	Sugar.	Total solids.	
				Gravimetric.	Babcock formula.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Oct. 23—a. m.	2.26	1.30	2.65	22.22	12.86
Oct. 24—a. m.	1.24	3.26	3.81	15.78	12.55
Oct. 24—p. m.	1.09	4.57	4.41	16.53	14.06
Oct. 25—a. m.	.98	5.42	4.89	17.19	15.04
Oct. 25—p. m.	.93	5.08	4.63	16.34	14.67
Oct. 26—a. m.	.87	6.23	4.79	17.04	16.01
Oct. 26—p. m.	.82	5.35	5.40	16.06	14.88
Oct. 27—a. m.	.79	5.87	5.53	16.85	15.53
Oct. 27—p. m.	.73	5.60	4.96	15.26	15.18

Table 5 was prepared from averages given in Table 3. The specific gravity of the plasma and the increase in specific gravity of the plasma caused by 1 per cent of plasma solids (Babcock's value *a*) were calculated by means of the Babcock formula. The specific gravity of the plasma solids was calculated by the formula of Fleischmann, to which reference has already been made. The last column of figures shows the factor which would result in each case were our figures for the value of *a* substituted for Babcock's in his formula.

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[1] Richmond's Dairy Chemistry, p. 65.

TABLE 5.—*Average data for each breed of cows.*

Breed.	Number of analyses.	Average specific gravity of milk.	Average fat content.	Average total solids.	Average specific gravity of plasma.	Average specific gravity of plasma solids.	Average value for *a*.	Factor.
			Per cent.	*Per cent.*				
Jersey	95	1.0335	4.99	14.30	1.03958	1.648	0.004052	2.468
Holstein	133	1.0314	3.09	11.48	1.03500	1.624	.004038	2.477
Ayrshire	124	1.0325	4.06	12.97	1.03734	1.637	.004025	2.485
Shorthorn	78	1.0336	3.75	12.80	1.03811	1.650	.004049	2.469
All breeds	430	1.0328	3.97	12.89	1.03752	1.638	.004044	2.470

It will be noted that there is no great variation in the figures in the last three columns, and also that the factor is but slightly different from Babcock's 2.5. This would, of course, follow from the close agreement between the grand average figures for the gravimetric total solids and that calculated by the Babcock formula.

EXPERIMENTS TO DETERMINE ACCURACY OF LACTOMETERS.

Having found which formula was best adapted for the purpose, the next question which naturally suggests itself is whether the ordinary lactometer when used to determine the specific gravity of milk is sufficiently accurate.

Thirteen lactometers were available for comparison; 11 of these were Quevenne lactometers and 2 were of the type known as the New York Board of Health lactometer. These were thought to represent fairly well the ordinary lactometers on the market. They were compared with the Westphal balance on three different samples of milk, with the following results:

TABLE 6.—*Showing comparisons of various lactometers with Westphal balance.*

Instrument.	Specific gravity of milk samples.			Instrument.	Specific gravity of milk samples.		
	Skim milk.	Holstein.	Holstein fresh.		Skim milk.	Holstein.	Holstein fresh.
Quevenne lactometer 1	1.0345	1.0315	1.0325	Quevenne lactometer 9	1.0350	1.0320	1.0330
Quevenne lactometer 2	1.0340	1.0310	1.0320	Quevenne lactometer 10	1.0335	1.0310	1.0318
Quevenne lactometer 3	1.0340	1.0315	1.0325	Quevenne lactometer 11	1.0350	1.0320	1.0330
Quevenne lactometer 4	1.0335	1.0300	1.0320	New York Board of Health lactometer 1	1.0328	1.0307	1.0319
Quevenne lactometer 5	1.0330	1.0300	1.0315	New York Board of Health lactometer 2	1.0299	1.0281	1.0290
Quevenne lactometer 6	1.0340	1.0310	1.0325	Westphal balance	1.0345	1.0313	1.0325
Quevenne lactometer 7	1.0360	1.0330	1.0340				
Quevenne lactometer 8	1.0370	1.0335	1.0350				

A glance at the foregoing figures will show discrepancies which are sufficient in some cases to account for as much as 1 per cent of total solids calculated from the Babcock formula. Of course, much of the discrepancy is due to the fault of the manufacturer in not properly calibrating the instruments. However, in none of the lactometers tested was the scale divided into less than whole Quevenne degrees. Fractions of degrees could be read only by interpolation, and then the divisions were generally so narrow that a closer interpolation than one-half of a degree was impossible; in fact, in some cases it was hardly possible to read closer than whole degrees. One Quevenne degree with a Babcock formula will account for 0.25 per cent of total solids. It is therefore obvious that the ordinary lactometer is unsuited for other than very gross results.

The sensitiveness of the hydrometer, or lactometer, as it is termed when made for the special purpose of determining the specific gravity of milk, depends upon the ratio of the size of the bulb to the diameter of the stem. The larger the bulb is in proportion to the diameter of the stem, the more sensitive will be the lactometer, or, in other words, the longer will be the spaces representing units on the scale. A lactometer, then, may be made more sensitive by either diminishing the size of the stem or by enlarging the bulb. But the smaller the stem the more fragile is the instrument, and the larger the bulb, the more cumbersome.

In designing a lactometer for our work several points were taken into account: (1) That it should accommodate the usual ranges of normal milk; (2) that it should have scale divisions representing tenths of Quevenne degrees; and (3) that it must be neither too fragile nor too cumbersome for practical use outside of a chemical laboratory.

After considerable experimenting in the laboratory the dimensions of an instrument were decided upon and several were made to order from our specifications. (See fig. 1.)

In order to test these lactometers against the Westphal balance, salt solutions were used, and the following results were obtained:

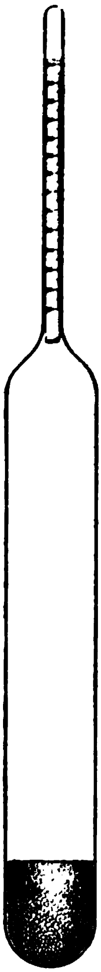

FIG. 1.—Lactometer designed for use in experimental work.

TABLE 7.—*Comparison of new lactometers with Westphal balance, using salt solutions.*

Solution.	Westphal.	Lactometer I.	Lactometer II.
I	1.0245	1.0248	1.0248
II	1.0283	1.0287	1.0287
III	1.0315	1.0318	1.0319
IV	1.0352	1.0351	1.0352

Little comment is required on the above figures, as it is seen that the results obtained with the lactometers are practically identical with those of the Westphal balance.

TESTS OF BABCOCK FORMULA AND NEW LACTOMETER WITH INDIVIDUAL MILKINGS.

Since the figures given in the first part of this bulletin were all based on results obtained on composite samples from individual cows, it was deemed desirable at this point to test the Babcock formula on milk from individual milkings and at the same time to compare the figures obtained by the new lactometers and the Westphal balance on the same milk. The only change in the laboratory procedure was that the percentage of fat was obtained by the Babcock test instead of by the extraction method.

Four cows were selected with which to make the tests under conditions comparable with those found in making official tests of dairy cattle. For this purpose pure-bred cows were used, representing four breeds. These cows were milked and fed three times daily—at 5 a. m., 1 p. m., and 8 p. m. The animals remained in the barn the greater part of the time. They were allowed the freedom of a lot from two to four hours in the forenoon and from one to two hours in the afternoon. Each animal was fed according to her individual capacity and characteristics. The cows were on official test at the time these samples were secured and were receiving such treatment as, in the judgment of the herdsman, would give the best results for this purpose. The rations consisted of corn, silage, alfalfa hay, corn meal, bran, oats, and oil meal in somewhat varying proportions. Table 8 gives more specific data regarding the four cows used. The duration of the test was seven days, the average yields of milk and of butter fat for this period being as follows:

TABLE 8.—*Milking records of cows used in tests.*

No. of Cow.	Breed.	Days in milk.	Average yield of milk per day.	Average yield of fat per day.
			Pounds.	*Pounds.*
16	Jersey	367	10.9	0.57
294	Holstein	119	16.7	.57
399	Ayrshire	20	13.7	.53
461	Shorthorn	258	10.5	.43

The results of the work on the individual milkings from the cows described in the above table are found in Tables 9, 10, 11, and 12, next following.

TABLE 9.—*Lactometer results on individual milkings from Holstein-Friesian cow No. 204.*

Sample No.	Specific gravity—			Fat.	Percentage of total solids—			
	Westphal balance.	Lactometer I.	Lactometer II.		Gravimetric.	Westphal.	Lactometer I.	Lactometer II.
				Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
L1	1.0310	1.0321	1.0320	2.9	11.59	11.23	11.52	11.49
L2	1.0320	1.0326	1.0324	3.2	12.26	11.85	12	11.95
L3	1.0315	1.0315	1.0312	4.3	12.93	13.05	13.05	12.97
L4	1.0308	1.0312	1.0310	3.5	11.91	11.91	12.01	11.96
L5	1.0317	1.0319	1.0317	2.9	11.57	11.41	11.46	11.41
L6	1.0316	1.0320	1.0318	3.8	12.72	12.47	12.57	12.52
L7	1.0336	1.0335	1.0336	3.4	12.70	12.49	12.47	12.49
L8	1.0321	1.0323	1.0324	3.3	12.23	12	12.05	12.07
L9	1.0299	1.0300	1.0301	3.5	12.32	11.69	11.71	11.74
L10	1.0320	1.0325	1.0322	3.5	12.26	12.21	12.34	12.26
L11	1.0341	1.0337	1.0335	2.93	11.95	12.08	11.93	11.93
L12	1.0318	1.0316	1.0314	4	12.62	12.76	12.71	12.66
L13	1.0318	1.0320	1.0320	3.3	12.07	11.92	11.97	11.97
L14	1.0327	1.0322	1.0322	2.75	11.39	11.49	11.36	11.36
L15	1.0303	1.0298	1.0298	4.2	12.84	12.63	12.50	12.50
L16	1.0304	1.0312	1.0308	4.23	13.17	12.71	12.91	12.81
L17	1.0327	1.0324	1.0322	2.8	11.59	11.55	11.47	11.42
L18	1.0312	1.0312	1.0309	3.95	12.60	12.55	12.55	12.48
L19	1.0313	1.0320	1.0317	3.70	12.61	12.28	12.45	12.38
L20	1.0313	1.0321	1.0319	3.35	12.07	11.86	12.08	12.01
L21	1.0310	1.0317	1.0317	3.78	12.44	12.32	12.50	12.50

TABLE 10.—*Lactometer results on individual milkings from Jersey cow No. 16.*

Sample No.	Specific gravity—			Fat.	Percentage of total solids—			
	Westphal balance.	Lactometer I.	Lactometer II.		Gravimetric.	Westphal.	Lactometer I.	Lactometer II.
				Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
L22	1.0325	1.0329	1.0327	5.2	14.92	14.39	14.49	14.44
L23	1.0346	1.0348	1.0348	5.1	14.97	14.79	14.84	14.84
L24	1.0325	1.0330	1.0329	5.6	15.45	14.88	15	14.98
L25	1.0342	1.0347	1.0347	4.88	14.65	14.45	14.58	14.58
L26	1.0346	1.0350	1.0348	4.6	14.38	14.19	14.30	14.24
L27	1.0333	1.0335	1.0333	5.18	14.89	14.59	14.64	14.59
L28	1.0340	1.0351	1.0349	5.25	15.25	14.82	15.11	15.05
L29	1.0332	1.0338	1.0336	5.20	14.56	14.56	14.71	14.66
L30	1.0327	1.0333	1.0333	5.68	15.33	15.05	15.20	15.20
L31	1.0345	1.0348	1.0349	5.40	15.28	15.14	15.21	15.24
L32	1.0355	1.0357	1.0355	5	14.85	14.91	14.96	14.91
L33	1.0344	1.0348	1.0346	5	14.87	14.62	14.72	14.67
L34	1.0340	1.0342	1.0341	5.23	14.86	14.82	14.87	14.85
L35	1.0350	1.0350	1.0348	4.93	14.62	14.72	14.72	14.66
L36	1.0330	1.0333	1.0330	5.75	15.50	15.18	15.26	15.18
L37	1.0351	1.0354	1.0354	5.15	14.93	14.99	15.06	15.06
L38	1.0343	1.0347	1.0346	5.25	15.09	14.90	15	14.97
L39	1.0332	1.0333	1.0335	5.55	15.20	14.99	15.02	15.07
L40	1.0351	1.0345	1.0344	5.20	15.19	15.05	14.89	14.86
L41	1.0354	1.0347	1.0346	5.25	15.26	15.18	15	14.97
L42	1.0333	1.0333	1.0333	5.30	15.59	14.71	14.71	14.71

TABLE 11.—*Lactometer results on individual milkings from Shorthorn cow No. 401.*

Sample No.	Specific gravity—			Fat.	Percentage of total solids—			
	Westphal balance.	Lactometer I.	Lactometer II.		Gravimetric.	Westphal.	Lactometer I.	Lactometer II.
				Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
L43	1.0340	1.0342	1.0342	3.8	13.44	13.08	13.13	13.13
L44	1.0344	1.0345	1.0344	3.65	13.22	13	13.03	13
L45	1.0340	1.0342	1.0342	4.5	14.43	13.92	13.97	13.97
L46	1.0350	1.0352	1.0351	3.9	13.80	13.45	13.50	13.48
L47	1.0352	1.0354	1.0353	3	12.76	12.41	12.46	12.44
L48	1.0340	1.0343	1.0342	4.4	14.29	13.80	13.88	13.85
L49	1.0344	1.0348	1.0347	4	13.54	13.42	13.52	13.50
L50	1.0357	1.0363	1.0363	3.7	13.82	13.39	13.54	13.54
L51	1.0335	1.0337	1.0336	4.8	14.31	14.16	14.21	14.18
L52	1.0308	1.0316	1.0317	3.55	11.92	11.97	12.17	12.20
L53	1.0355	1.0360	1.0360	2.95	12.67	12.43	12.55	12.55
L54	1.0344	1.0351	1.0349	4.45	13.59	13.96	14.15	14.09
L55	1.0347	1.0348	1.0345	4.50	14.14	14.10	14.12	14.05
L56	1.0349	1.0352	1.0350	4.45	13.92	14.09	14.17	14.12
L57	1.0337	1.0339	1.0338	5	14.48	14.46	14.49	14.47
L58	1.0335	1.0339	1.0338	4.65	14.23	13.98	14.07	14.05
L59	1.0350	1.0355	1.0354	3.60	12.73	13.09	13.22	13.19
L60	1.0362	1.0360	1.0359	4.60	14.56	14.60	14.55	14.52
L61	1.0355	1.0359	1.0360	4.40	14.37	14.19	14.28	14.31
L62	1.0358	1.0363	1.0364	4.15	14.04	13.96	14.09	14.11
L63	1.0344	1.0351	1.0349	4.30	14.	13.78	13.97	13.91

TABLE 12.—*Lactometer results on individual milkings from Ayrshire cow No. 300.*

Sample No.	Specific gravity—			Fat.	Percentage of total solids—			
	Westphal balance.	Lactometer I.	Lactometer II.		Gravimetric.	Westphal.	Lactometer I.	Lactometer II.
				Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
L64	1.0320	1.0327	1.0325	3	11.52	11.61	11.79	11.74
L65	1.0300	1.0303	1.0301	5.10	13.38	13.63	13.71	13.66
L66	1.0322	1.0323	1.0321	2.50	10.79	11.05	11.08	11.03
L67	1.0325	1.0327	1.0327	4.05	12.99	13	13.06	13.06
L68	1.0326	1.0331	1.0328	2.65	11.21	11.33	11.47	11.38
L69	1.0304	1.0310	1.0308	4.25	12.40	12.71	12.86	12.75
L70	1.0315	1.0315	1.0313	2.75	11.01	11.18	11.18	11.13
L71	1.0317	1.0323	1.0322	3.10	11.72	11.66	11.81	11.78
L72	1.0313	1.0309	1.0305	3.15	11.45	11.62	11.51	11.42
L73	1.0295	1.0294	1.0294	5	13.42	13.39	13.36	13.36
L74	1.0308	1.0310	1.0308	3.25	11.53	11.61	11.66	11.61
L75	1.0320	1.0322	1.0319	4.25	13.41	13.12	13.17	13.09
L76	1.0315	1.0317	1.0316	4.90	13.61	13.77	13.82	13.79
L77	1.0327	1.0327	1.0327	3.80	12.49	12.75	12.75	12.75
L78	1.0320	1.0322	1.0320	4.90	13.67	13.90	13.95	13.90
L79	1.0322	1.0324	1.0322	4.50	13.54	13.47	13.52	13.47
L80	1.0326	1.0329	1.0326	3.75	12.06	12.66	12.74	12.66
L81	1.0295	1.0290	1.0287	4.75	12.48	13.09	12.96	12.89
L82	1.0330	1.0328	1.0328	3.30	12.	12.22	12.17	12.17
L83	1.0296	1.0298	1.0297	4.25	12.51	12.51	12.56	12.54
L84	1.0278	1.0280	1.0278	5.40	13.15	13.44	13.49	13.44

EFFECT OF TEMPERATURE ON SPECIFIC GRAVITY OF MILK.

An increase in temperature is accompanied by a lowering of the specific gravity as determined by the lactometer. To show the importance of maintaining the proper temperature when using the lactometer and at the same time to determine the size of the error introduced by the difference of a few degrees in temperature, specific gravity determinations were made at different temperatures on several samples of milk. Only ordinary precautions were taken in this experiment, and the results are about such as would be obtained were the lactometers in practical use.

Samples of milk were taken from representatives of the four breeds of cows previously used.

TABLE 13.—*Effect of temperature on specific gravity of milk when determined with new lactometer.*

Temperatures.	Jersey milk.		Shorthorn milk.		Ayrshire milk.		Holstein-Friesian milk.	
	Specific gravity.	Difference.	Specific gravity.	Difference.	Specific gravity.	Difference.	Specific gravity.	Difference.
9° C	1.0364	....	1.0365	....	1.0330	....	1.0291	....
11° C	1.0360	0.0004	1.0361	0.0004	1.0327	0.0003	1.0287	0.0004
13° C	1.0356	.0004	1.0357	.0004	1.0323	.0004	1.0283	.0004
15° C	1.0351	.0005	1.0352	.0005	1.0318	.0005	1.0279	.0004
17° C	1.0345	.0006	1.0347	.0005	1.0313	.0005	1.0275	.0004
19° C	1.0338	.0007	1.0341	.0006	1.0308	.0005	1.0270	.0005
21° C	1.0331	.0007	1.0335	.0006	1.0302	.0006	1.0265	.0005
Average	....	.0006	....	.0005	....	.0005	....	.0004

TABLE 14.—*Showing composition of milk used in Table 13.*

Milk.	Fat.	Total solids.
	Per cent.	*Per cent.*
Jersey	5.2	15.21
Shorthorn	4	13.78
Ayrshire	3.7	12.28
Holstein-Friesian	2.7	10.02

It will at once be seen that the variation is different in different samples and also at different temperatures with the same sample. The greatest variation is in the Jersey, milk where the percentage of total solids is highest, and least in the Holstein-Friesian milk, where the percentage of total solids is lowest. As the temperature rises the variation for each degree increases.

The average variation per centigrade degree counting all four breeds, is 0.00025, which would account for an error of about 0.08 per cent total solids if calculated with the Babcock formula. Reduced to Fahrenheit degrees the error would be five-ninths of 0.08, or about 0.044 per cent for each degree.

RECKNAGEL'S PHENOMENON.

Milk when freshly drawn contains numerous bubbles of gas, and it is not until these have disappeared that the specific gravity can be determined. It has been demonstrated by Recknagel[1] and confirmed by other investigators that the specific gravity of milk changes on standing. On taking the specific gravity of milk after the air bubbles had escaped and again several hours later he found an increase. This peculiarity is called the Recknagel phenomenon. He ascribes the increase to a change in the volume of the proteins. The increase begins two or three hours after milking, and if the milk is held at about 15° C. continues with decreasing rapidity for two days. The amount of the increase is between 0.0008 and 0.0015, depending on the richness of the milk. This change is accelerated by lower temperatures, and the normal specific gravity, or the point where no further change takes place, may be obtained by keeping the milk at 5° C. or lower for six hours.

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[1] Milchzeitung, Band 12, p. 419, Bremen, 1883.

HOW TO USE THE MODIFIED LACTOMETER AND TABLE.

This section is designed to assist those who may desire to make use of the modified lactometer described in the preceding section and who are not accustomed to using delicate lactometers.

Materials required: 1. The lactometer. 2. A pan of warm water. 3. An accurate dairy thermometer. 4. A suitable cylinder to contain the sample while making the reading.

The cylinder may be made of tin or copper and should have the following dimensions: Inside diameter, 1¾ inches; height, 13 inches. To prevent it from tipping over it should have a base of the same material about 2¾ inches in diameter.

METHOD.

Immediately after milking the milk should be thoroughly mixed and a sample of about 1 pint placed in a cream bottle. This should then be put into the refrigerator and kept there for ten or twelve hours, or until the next milking. It is then removed from the refrigerator and again well mixed by pouring back and forth several times from the bottle into another bottle or cup. At this point care must be taken not to mix air with the milk. This can be avoided by pouring against the sides of the receptacle to prevent foaming. After mixing, the bottle is placed in a pan of warm water and heated while being constantly stirred with the thermometer until the temperature reaches 60° F. The milk is then poured into the cylinder, which should also have been warmed in the pan so that it will not cool the milk. The lactometer is now quickly lowered into the milk, of which there should be a sufficient quantity in the cylinder to overflow it, and allowed to come to rest. The point on the graduated scale which is at the same level as the surface of the milk is then read. This reading gives Quevenne degrees, which may be converted into specific gravity if desired by dividing by 1,000 and then adding 1 to the quotient.

Owing to the tendency of the milk to form a meniscus about the stem of the lactometer, it is impossible to read directly the exact point on the scale that is at the same level as the surface of the milk. A safe rule for obtaining a very close approximation to the correct figure is to add 0.2 to the reading taken where the top of the meniscus strikes the scale. For example, if the scale reads 31.8 at the top of the meniscus, the corrected reading in Quevenne degrees would be 32 and the specific gravity 1.032.

Care must be taken that the temperature of the milk when the lactometer is read is exactly 60° F.; otherwise a very considerable error will be introduced. After using the lactometer it should be rinsed in clean water, wiped dry, and restored to its case.

The percentage of fat should be determined by the Babcock test either on the sample used for the specific gravity determination or on another taken at the same time.

Having by this procedure found the specific gravity of the milk and the percentage of fat, the total solids can be found by referring to Table 15, which is a modified form of one published by Babcock.[1] In our table the percentage of total solids is given. If percentage of plasma solids is wanted, it can be found by subtracting the percentage of fat from the percentage of total solids.

DIRECTIONS FOR USING THE TABLE.

If the specific gravity as expressed in Quevenne degrees is a whole number, the percentage of total solids is found at the intersection of the vertical column headed by this number with the horizontal column corresponding to the percentage of fat.

If the specific gravity as expressed in Quevenne degrees is a whole number and a decimal, the percentage of total solids corresponding to the whole number is first found and to this is added the fraction found opposite the tenth under "Proportional parts." Two examples may suffice for illustration: (1) Fat, 3.8 per cent; specific gravity, 32. Under column headed 32 we find 12.57 per cent, corresponding to 3.8 per cent fat. (2) Fat, 3.8 per cent; specific gravity, 32.5. The percentage of total solids corresponding to this percentage of fat and a specific gravity of 32 is 12.57. Under "Proportional parts" the fraction 0.13 appears opposite 0.5. This added to 12.57 makes 12.70, which is the desired percentage.

An inspection of the table shows that the percentage of total solids increases practically at the rate of 0.25 for each lactometer degree and 1.2 for each per cent of fat. This gives rise to Babcock's simpler formula

$$\text{Total solids} = \tfrac{1}{4} L + 1.2 f$$

(L = lactometer reading in Quevenne degrees, and f = percentage fat).

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[1] Twelfth Annual Report of the Wisconsin Agricultural Experiment Station, p. 124.

This simple formula can be used in cases not provided for in the table, and the error introduced will be inconsiderable.

TABLE 15.—*Table for determining total solids in milk from any given specific gravity and percentage of fat.*

Percentage of fat.	Lactometer reading at 60° F. (Quevenne degrees).										
	26	27	28	29	30	31	32	33	34	35	36
	Per cent total solids.	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*
2.00	8.90	9.15	9.40	9.65	9.90	10.15	10.40	10.66	10.91	11.16	11.41
2.05	8.96	9.21	9.46	9.71	9.96	10.21	10.46	10.72	10.97	11.22	11.47
2.10	9.02	9.27	9.52	9.77	10.02	10.27	10.52	10.78	11.03	11.28	11.53
2.15	9.08	9.33	9.58	9.83	10.08	10.33	10.58	10.84	11.09	11.34	11.59
2.20	9.14	9.39	9.64	9.89	10.14	10.39	10.64	10.90	11.15	11.40	11.65
2.25	9.20	9.45	9.70	9.95	10.20	10.45	10.70	10.96	11.21	11.46	11.71
2.30	9.26	9.51	9.76	10.01	10.26	10.51	10.76	11.02	11.27	11.52	11.77
2.35	9.32	9.57	9.82	10.07	10.32	10.57	10.82	11.08	11.33	11.58	11.83
2.40	9.38	9.63	9.88	10.13	10.38	10.63	10.88	11.14	11.39	11.64	11.89
2.45	9.44	9.69	9.94	10.19	10.44	10.69	10.94	11.20	11.45	11.70	11.95
2.50	9.50	9.75	10.00	10.25	10.50	10.75	11.00	11.26	11.51	11.76	12.01
2.55	9.56	9.81	10.06	10.31	10.56	10.81	11.06	11.32	11.57	11.82	12.07
2.60	9.62	9.87	10.12	10.37	10.62	10.87	11.12	11.38	11.63	11.88	12.13
2.65	9.68	9.93	10.18	10.43	10.68	10.93	11.18	11.44	11.69	11.94	12.19
2.70	9.74	9.99	10.24	10.49	10.74	10.99	11.24	11.50	11.75	12.00	12.25
2.75	9.80	10.05	10.30	10.55	10.80	11.05	11.31	11.56	11.81	12.06	12.31
2.80	9.86	10.11	10.36	10.61	10.86	11.11	11.37	11.62	11.87	12.12	12.37
2.85	9.92	10.17	10.42	10.67	10.92	11.17	11.43	11.68	11.93	12.18	12.43
2.90	9.98	10.23	10.48	10.73	10.98	11.23	11.49	11.74	11 99	12.24	12.49
2.95	10.04	10.29	10.54	10.79	11.04	11.30	11.55	11.80	12.05	12.30	12.55
3.00	10.10	10.35	10.60	10.85	11.10	11.36	11.61	11.86	12.11	12.36	12.61
3.05	10.16	10.41	10.66	10.91	11.17	11 42	11.67	11.92	12.17	12.42	12.68
3.10	10.22	10.47	10.72	10.97	11.23	11.48	11.73	11.98	12.23	12.48	12.74
3.15	10.28	10.53	10.78	11.03	11.29	11.54	11.79	12.04	12.29	12.55	12.80
3.20	10.34	10.59	10.84	11.09	11.35	11.60	11.85	12.10	12.35	12.61	12.86
3.25	10.40	10.65	10.90	11.16	11.41	11.66	11.91	12.16	12.42	12.67	12.92
3.30	10.46	10.71	10.96	11.22	11.47	11.72	11.97	12.22	12.48	12.73	12.98
3.35	10.52	10.77	11.03	11.28	11.53	11.78	12.03	12.28	12.54	12.79	13.04
3.40	10.58	10.83	11.09	11.34	11.59	11.84	12.09	12.34	12.60	12.85	13.10
3.45	10.64	10.89	11.15	11.40	11.65	11.90	12.15	12.40	12.66	12.91	13.16
3.50	10.70	10.95	11.21	11 46	11.71	11.96	12.21	12.46	12.72	12.97	13.22
3.55	10.76	11.02	11.27	11.52	11.77	12.02	12.27	12.52	12.78	13.03	13.28
3.60	10.82	11.08	11.33	11.58	11.83	12.08	12.33	12.58	12.84	13.09	13.34
3.65	10.88	11.14	11.39	11.64	11.89	12.14	12.39	12.64	12.90	13.15	13.40
3.70	10.94	11.20	11.45	11.70	11.95	12.20	12.45	12.70	12.96	13.21	13.46
3.75	11.00	11.26	11.51	11.76	12.01	12.26	12.51	12.76	13.02	13.27	13.52
3.80	11.06	11.32	11.57	11.82	12.07	12.32	12.57	12.82	13.08	13.33	13.58
3.85	11.12	11.38	11.63	11.88	12.13	12.38	12.63	12.88	13.14	13.39	13.64
3.90	11.18	11.44	11.69	11.94	12.19	12.44	12.69	12.94	13.20	13.45	13.70
3.95	11.24	11.50	11.75	12.00	12.25	12.50	12.75	13.00	13.26	13.51	13.77
4.00	11.30	11.56	11.81	12.06	12.31	12.56	12.81	13.06	13.32	13.57	13.83
4.05	11.36	11.62	11.87	12.12	12.37	12.62	12.87	13.12	13.38	13.63	13.89
4.10	11.42	11.68	11.93	12.18	12.43	12.68	12.93	13.18	13.44	13.69	13.95
4.15	11.48	11.74	11.99	12.24	12.49	12.74	12.99	13.25	13.50	13.76	14.01
4.20	11.54	11.80	12.05	12.30	12.55	12.80	13.05	13.31	13.56	13.82	14.07
4.25	11.60	11.86	12.11	12.36	12.61	12.86	13.12	13.37	13.62	13.88	14.13
4.30	11.66	11.92	12.17	12.42	12.67	12.92	13.18	13.43	13.68	13.94	14.19
4.35	11.72	11.98	12.23	12.48	12.73	12.98	13 24	13.49	13.74	14.00	14.25
4.40	11.78	12.04	12.29	12.54	12.79	13.04	13.30	13.55	13.80	14.06	14.31
4.45	11.84	12.10	12.35	12.60	12.85	13.10	13.36	13.61	13.86	14.12	14.37
4.50	11.90	12.16	12.41	12.66	12.91	13.16	13.42	13.67	13.92	14.18	14.43
4.55	11.97	12.22	12.47	12.72	12.97	13.22	13.48	13.73	13.98	14.24	14.49
4.60	12.03	12.28	12.53	12.78	13.03	13.28	13.54	13.79	14.04	14.30	14.55
4.65	12.09	12.34	12.59	12.84	13.09	13.34	13.60	13.85	14.10	14.36	14.61
4.70	12.15	12.40	12.65	12.90	13.15	13.40	13.66	13.91	14.16	14.42	14.67
4.75	12.21	12.46	12.71	12.96	13.21	13.46	13.72	13.97	14.22	14.48	14.73
4.80	12.27	12.52	12.77	13.02	13.27	13.52	13.78	14.03	14.28	14.54	14.79
4.85	12.33	12.58	12.83	13.08	13.33	13.58	13.84	14.09	14.34	14.60	14.85
4.90	12.39	12.64	12.89	13.14	13.39	13.64	13.90	14.15	14.40	14.66	14.91
4.95	12.45	12.70	12.95	13.20	13.45	13.70	13.96	14.21	14.46	14.72	14.97

TABLE 15.—*Table for determining total solids in milk from any given specific gravity and percentage of fat*—Continued.

Per centage of fat.	Lactometer reading at 60° F. (Quevenne degrees).										
	26	27	28	29	30	31	32	33	34	35	36
	Per cent total solids.	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*	*Per cent total solids.*
5.00	12.51	12.76	13.01	13.26	13.51	13.76	14.02	14.27	14.52	14.78	15.03
5.05	12.57	12.82	13.07	13.32	13.57	13.83	14.08	14.33	14.58	14.84	15.09
5.10	12.63	12.88	13.13	13.38	13.63	13.89	14.14	14.39	14.64	14.90	15.15
5.15	12.69	12.94	13.19	13.44	13.69	13.95	14.20	14.45	14.70	14.96	15.21
5.20	12.75	13.00	13.25	13.50	13.75	14.01	14.26	14.51	14.76	15.02	15.27
5.25	12.81	13.06	13.31	13.56	13.81	14.07	14.32	14.57	14.82	15.08	15.33
5.30	12.87	13.12	13.37	13.62	13.87	14.13	14.38	14.63	14.88	15.14	15.39
5.35	12.93	13.18	13.43	13.68	13.93	14.19	14.44	14.70	14.95	15.20	15.45
5.40	12.99	13.24	13.49	13.74	14.00	14.25	14.50	14.76	15.01	15.26	15.51
5.45	13.05	13.30	13.55	13.80	14.06	14.31	14.56	14.82	15.07	15.32	15.57
5.50	13.11	13.36	13.61	13.86	14.12	14.37	14.62	14.88	15.13	15.38	15.63
5.55	13.17	13.42	13.67	13.93	14.18	14.43	14.69	14.94	15.19	15.44	15.69
5.60	13.23	13.48	13.73	13.99	14.24	14.49	14.75	15.00	15.25	15.50	15.75
5.65	13.29	13.54	13.79	14.05	14.30	14.55	14.81	15.06	15.31	15.56	15.81
5.70	13.35	13.60	13.85	14.11	14.36	14.61	14.87	15.12	15.37	15.62	15.87
5.75	13.41	13.66	13.91	14.17	14.42	14.68	14.93	15.18	15.43	15.68	15.93
5.80	13.47	13.72	13.97	14.23	14.48	14.74	14.99	15.24	15.49	15.74	15.99
5.85	13.53	13.78	14.04	14.29	14.54	14.80	15.05	15.30	15.55	15.80	16.06
5.90	13.59	13.84	14.10	14.35	14.60	14.86	15.11	15.36	15.61	15.86	16.12
5.95	13.65	13.90	14.16	14.41	14.66	14.92	15.17	15.42	15.67	15.92	16.18
6.00	13.71	13.96	14.22	14.47	14.72	14.98	15.23	15.48	15.73	15.98	16.24
6.05	13.77	14.02	14.28	14.53	14.78	15.04	15.29	15.54	15.79	16.04	16.30
6.10	13.83	14.08	14.34	14.59	14.84	15.10	15.35	15.60	15.85	16.10	16.35
6.15	13.89	14.14	14.40	14.65	14.90	15.16	15.41	15.66	15.91	16.16	16.42
6.20	13.95	14.20	14.46	14.71	14.96	15.22	15.47	15.72	15.97	16.22	16.48
6.25	14.01	14.26	14.52	14.77	15.02	15.28	15.53	15.78	16.03	16.28	16.54
6.30	14.07	14.32	14.58	14.83	15.08	15.34	15.59	15.84	16.09	16.34	16.60
6.35	14.13	14.38	14.64	14.90	15.14	15.40	15.65	15.90	16.15	16.40	16.66
6.40	14.19	14.44	14.70	14.96	15.20	15.46	15.71	15.96	16.21	16.46	16.72
6.45	14.25	14.50	14.76	15.02	15.26	15.52	15.77	16.02	16.27	16.52	16.78
6.50	14.31	14.56	14.82	15.08	15.32	15.58	15.83	16.08	16.33	16.58	16.84
6.55	14.37	14.62	14.88	15.14	15.38	15.64	15.89	16.14	16.39	16.64	16.90
6.60	14.43	14.68	14.94	15.20	15.44	15.70	15.95	16.20	16.45	16.70	16.96
6.65	14.49	14.74	15.00	15.26	15.50	15.76	16.01	16.26	16.51	16.76	17.02
6.70	14.55	14.80	15.06	15.32	15.56	15.82	16.07	16.32	16.57	16.82	17.08
6.75	14.61	14.86	15.12	15.38	15.62	15.88	16.13	16.38	16.63	16.88	17.14
6.80	14.67	14.92	15.18	15.44	15.68	15.94	16.19	16.44	16.69	16.94	17.20
6.85	14.73	14.98	15.24	15.50	15.74	16.00	16.25	16.50	16.75	17.00	17.26
6.90	14.79	15.04	15.30	15.56	15.80	16.06	16.31	16.56	16.81	17.06	17.32
6.95	14.85	15.10	15.36	15.62	15.86	16.12	16.37	16.62	16.87	17.12	17.38

PROPORTIONAL PARTS.

Lactometer fraction.	Fraction to be added to total solids.	Lactometer fraction.	Fraction to be added to total solids.	Lactometer fraction.	Fraction to be added to total solids.
0.1	0.03	0.4	0.10	0.7	0.18
.2	.05	.5	.13	.8	.20
.3	.08	.6	.15	.9	.23

SUMMARY AND CONCLUSIONS.

1. For purposes where exact percentages of total solids are demanded the use of any formula will not fulfill the requirements.

2. Of the formulas in general use that known as the Babcock (revised) formula gave results closest to those obtained gravimetrically. In 430 composite samples analyzed for total solids 256, or nearly 60 per cent, when calculated with this formula agreed within 0.25 per cent of the figures obtained gravimetrically, and 389, or over 90 per cent, agreed within 0.50 per cent. In another test with 84 samples of milk obtained from four individual cows under official testing conditions, determinations made on each milking showed that the total solids calculated by the formula in 53 cases, or 63 per cent of the total, agreed within 0.25 per cent of the gravimetrically determined figures; and in 78 cases, or 93 per cent of the total, they agreed within 0.50 per cent.

3. Neither individuality nor breed in the cows seemed to exert any notable influence upon the application of the formulas.

4. The Babcock formula may be safely used with normal milk where only comparatively close approximations are required. It must be left to the decision of those in need of such figures as to whether or not the formula will fulfill their particular requirement.

5. The lactometers in common use for determining specific gravity of milk are not sufficiently sensitive to be used in connection with the Babcock fat test for estimating total solids in milk by formula. A modification of the Quevenne lactometer was, however, devised which was found to yield results quite as accurate as those obtained with the Westphal balance and at the same time so constructed that it may be used successfully by those unskilled in the use of chemical apparatus.

APPENDIX.

Table 16.—*Comparative determinations of total solids in milk.*

Test No.	Cow No.	Specific gravity (Quevenne degrees).	Fat.	Total solids.			
				Gravimetric.	Babcock.	Richmond.	Fleischmann.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	4	33.2	5.07	14.39	14.38	14.51	14.65
2	4	34.5	4.66	14.40	14.22	14.40	14.48
3	4	33.7	5.06	14.54	14.50	14.68	14.76
4	4	33.0	4.93	14.41	14.17	14.27	14.43
5	4	33.6	4.83	14.18	14.20	14.27	14.46
6	4	34.5	4.80	14.26	14.39	14.52	14.65
7	4	34.0	5.02	14.31	14.52	14.68	14.79
8	4	33.7	4.83	13.99	14.22	14.27	14.48
9	4	33.0	5.14	14.18	14.42	14.51	14.68
10	4	34.0	5.00	14.11	14.50	14.63	14.76
11	4	31.5	4.97	13.98	13.84	14.02	14.10
12	4	33.5	4.68	13.85	13.99	14.15	14.25
13	4	32.5	4.77	13.88	13.85	14.02	14.11
14	4	33.0	4.50	13.76	13.65	13.79	13.91
15	4	32.2	4.38	13.48	13.31	13.42	13.57
16	4	33.1	4.44	13.61	13.60	13.67	13.87
17	4	33.0	4.26	14.23	13.36	13.55	13.63
18	4	33.6	4.57	13.96	13.88	14.03	14.15
19	4	33.0	4.65	13.29	13.83	14.08	14.09
20	4	34.0	4.85	14.03	14.32	14.51	14.58
21	4	34.0	5.15	14.70	14.68	14.87	14.94
22	4	34.0	4.82	14.55	14.28	14.39	14.55
23	4	32.8	5.39	14.75	14.67	14.87	14.93
24	4	34.0	5.68	14.80	15.32	15.47	15.58
25	4	33.6	5.61	15.15	15.13	15.28	15.40
26	99	33.3	4.37	13.09	13.57	13.79	13.83
27	99	33.4	4.55	13.54	13.81	14.08	14.07
28	99	33.0	4.51	13.49	13.66	13.79	13.93
29	99	34.0	4.53	13.48	13.94	14.03	14.20
30	99	32.6	4.14	12.72	13.12	13.18	13.38
31	99	32.3	4.50	13.20	13.48	13.66	13.74
32	99	32.7	3.86	12.87	12.81	12.94	13.07
33	99	32.9	4.74	13.50	13.91	14.03	14.18
34	99	32.4	4.50	13.04	13.50	13.66	13.76
35	99	32.0	4.39	12.84	13.27	13.42	13.53
36	99	33.0	4.32	12.95	13.43	13.55	13.70
37	99	32.7	4.31	12.88	13.35	13.42	13.61
38	99	31.0	4.28	12.99	12.89	13.06	13.15
39	99	31.8	4.32	12.87	13.13	13.30	13.40
40	99	32.2	4.23	12.84	13.13	13.18	13.39
41	99	30.5	4.43	12.90	12.94	13.05	13.20
42	99	31.0	3.90	12.42	12.43	12.58	12.69
43	99	31.0	4.27	13.04	12.87	13.06	13.14
44	99	30.9	3.93	12.67	12.44	12.58	12.70
45	99	32.0	4.30	12.50	13.16	13.30	13.42
46	99	33.0	4.42	13.13	13.55	13.67	13.82
47	99	33.0	4.07	12.95	13.13	13.31	13.40
48	99	34.0	4.37	13.13	13.74	13.91	14.01
49	99	32.5	4.80	13.34	13.89	14.02	14.15
50	99	32.7	4.70	13.66	13.82	13.90	14.08
51	99	32.0	4.63	13.26	13.56	13.66	13.82
52	99	32.4	4.74	13.85	13.79	13.90	14.05
53	99	35.5	4.71	14.00	14.53	14.64	14.79
54	99	34.5	5.08	14.55	14.72	14.88	14.98
55	99	32.5	5.39	14.66	14.59	14.74	14.86
56	99	34.0	5.26	14.83	14.81	14.99	15.08
57	99	35.0	5.78	15.32	15.69	15.84	15.95
58	99	36.0	6.00	15.61	16.20	16.32	16.46
59	99	32.3	5.42	14.85	14.58	14.74	14.84
60	99	34.6	5.99	15.89	15.84	15.96	16.10
61	99	33.5	6.15	16.11	15.76	15.95	16.02

TABLE 16.—*Comparative determinations of total solids in milk*—Continued.

Test No.	Cow No.	Specific gravity (Quevenne degrees).	Fat.	Total solids.			
				Gravimetric.	Babcock.	Richmond.	Fleischmann.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
62	99	34.9	6.07	16.24	16.01	16.20	16.27
63	118	30.0	5.06	13.93	13.57	13.77	13.83
64	118	31.0	5.10	14.19	13.87	14.02	14.13
65	118	29.7	5.77	14.12	14.35	14.48	14.61
66	118	32.0	5.58	14.55	14.70	14.86	14.96
67	118	34.0	5.80	15.10	15.46	15.59	15.72
68	118	35.0	5.60	15.40	15.47	15.60	15.73
69	118	34.5	5.69	15.41	15.45	15.60	15.72
70	118	34.5	5.76	15.30	15.54	15.72	15.80
71	118	34.2	5.44	14.71	15.08	15.11	15.34
72	118	34.0	5.86	15.28	15.53	15.71	15.80
73	118	34.5	5.87	15.49	15.67	15.84	15.93
74	118	34.5	6.10	15.50	15.95	16.08	16.20
75	118	35.0	5.95	16.12	15.89	16.08	16.15
76	118	35.0	5.28	15.35	15.09	15.24	15.35
77	118	34.0	5.86	15.47	15.53	15.71	15.80
78	118	35.0	5.37	15.36	15.19	15.36	15.46
79	118	33.8	5.41	15.20	14.94	15.11	15.21
80	118	34.0	4.89	14.55	14.37	14.51	14.63
81	118	33.4	4.99	14.06	14.34	14.51	14.60
82	118	32.0	4.51	13.98	13.41	13.54	13.68
83	118	35.0	4.83	14.83	14.55	14.64	14.81
84	118	34.2	5.00	14.53	14.55	14.63	14.81
85	118	34.2	4.61	14.33	14.08	14.15	14.35
86	118	35.4	5.15	14.65	15.03	15.24	15.29
87	118	35.0	4.57	14.29	14.23	14.40	14.50
88	118	35.0	5.21	14.55	15.00	15.12	15.26
89	118	36.0	4.86	14.93	14.83	15.00	15.09
90	118	34.5	5.54	15.25	15.27	15.36	15.54
91	118	37.0	5.49	15.58	15.84	15.97	16.10
92	118	35.0	5.74	16.30	15.64	15.72	15.90
93	118	35.0	5.42	15.75	15.25	15.36	15.52
94	118	39.5	5.14	16.26	16.04	16.14	16.30
95	118	33.5	6.29	16.18	15.92	16.07	16.19
96	205	34.0	3.19	12.08	12.33	12.47	12.59
97	205	34.0	2.69	11.32	11.73	11.87	11.99
98	205	34.0	3.30	10.95	12.46	12.59	12.72
99	205	34.0	3.05	11.88	12.16	12.35	12.42
100	205	32.5	3.14	11.80	11.89	11.98	12.16
101	205	33.0	3.38	11.59	12.31	12.47	12.57
102	205	31.4	3.00	11.03	11.45	11.62	11.71
103	205	32.0	2.81	12.00	11.37	11.50	11.64
104	205	34.5	3.01	11.67	12.24	12.36	12.50
105	205	33.0	3.17	11.66	12.05	12.23	12.32
106	205	31.5	3.07	11.46	11.56	11.74	11.82
107	205	32.5	3.10	11.95	11.85	11.98	12.11
108	205	33.0	3.10	11.64	11.97	12.11	12.23
109	205	33.5	3.58	12.45	12.67	12.83	12.93
110	205	31.4	3.21	11.76	11.70	11.86	11.97
111	205	32.1	3.33	12.20	12.02	12.10	12.29
112	205	31.2	3.34	11.95	11.81	11.86	12.07
113	205	32.5	3.32	11.96	12.11	12.22	12.37
114	205	34.0	3.17	12.34	12.30	12.47	12.57
115	205	33.7	3.01	11.66	12.04	12.11	12.30
116	205	32.8	3.30	11.70	12.16	12.35	12.42
117	205	33.0	2.87	11.77	11.69	11.87	11.96
118	205	32.5	3.06	11.96	11.80	11.98	12.06
119	205	33.0	3.54	12.19	12.50	12.59	12.76
120	205	32.0	3.21	11.76	11.85	11.98	12.12
121	205	32.0	3.38	12.13	12.06	12.22	12.32
122	205	34.0	2.89	11.83	11.97	12.11	12.23
123	205	33.0	3.26	11.99	12.16	12.35	12.43
124	205	32.7	3.19	12.10	12.00	12.10	12.27
125	205	32.7	3.38	12.25	12.23	12.34	12.50
126	205	34.6	3.15	12.31	12.43	12.60	12.69
127	205	33.6	3.17	12.06	12.20	12.35	12.47
128	205	32.5	3.28	12.04	12.06	12.22	12.33
129	205	33.0	2.94	11.74	11.78	11.87	12.04
130	205	32.5	3.23	12.01	12.00	12.10	12.27
131	205	32.2	3.36	12.10	12.08	12.22	12.35
132	205	31.7	3.38	12.15	11.98	12.10	12.24
133	205	32.5	3.26	12.20	12.04	12.22	12.30
134	205	33.0	3.25	11.90	12.15	12.35	12.41
135	205	33.0	3.27	12.27	12.17	12.35	12.44
136	205	33.7	3.22	12.42	12.29	12.35	12.55
137	205	32.0	3.50	12.19	12.20	12.34	12.64
138	205	33.5	3.74	12.71	12.86	12.95	13.13

Table 16.—*Comparative determinations of total solids in milk*—Continued.

Test No.	Cow No.	Specific gravity (Quevenne degrees).	Fat.	Total solids.			
				Gravimetric.	Babcock.	Richmond.	Fleischmann.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
139	205	33.6	3.20	12.70	12.24	12.35	12.50
140	205	33.5	3.63	12.96	12.73	12.83	12.99
141	205	33.4	3.40	11.64	12.43	12.59	12.84
142	205	32.6	3.82	12.99	12.73	12.82	13.00
143	206	31.5	3.48	11.97	12.05	12.22	12.31
144	206	29.0	3.17	11.04	11.05	11.24	11.32
145	206	28.2	2.58	9.95	10.15	10.27	10.41
146	206	29.0	3.06	10.84	10.92	11.12	11.18
147	206	29.2	2.63	10.10	10.46	10.52	10.72
148	206	29.0	2.76	10.04	10.56	10.76	10.82
149	206	29.0	2.29	9.62	10.00	10.16	10.26
150	206	29.5	2.70	11.02	10.62	10.76	10.88
151	206	30.0	2.58	10.04	10.60	10.77	10.86
152	206	27.5	2.94	10.23	10.40	10.51	10.66
153	206	28.2	2.76	9.99	10.36	10.51	10.62
154	206	27.5	2.96	10.27	10.43	10.63	10.69
155	206	29.0	2.71	10.57	10.50	10.64	10.76
156	206	30.0	3.10	10.57	11.22	11.37	11.48
157	206	30.0	2.72	10.43	10.76	10.89	11.03
158	206	29.0	2.94	10.67	10.78	10.88	11.04
159	206	29.0	2.78	10.49	10.59	10.76	10.85
160	206	29.0	3.06	10.40	10.92	11.12	11.18
161	206	30.0	3.13	10.86	11.26	11.37	11.52
162	206	27.8	3.01	10.80	10.56	10.75	10.82
163	206	28.6	3.05	10.87	10.81	10.99	11.07
164	206	28.1	3.05	10.82	10.69	10.87	10.94
165	206	29.0	3.08	10.83	10.95	11.12	11.21
166	206	29.0	3.01	10.92	10.86	11.00	11.12
167	206	29.0	2.39	10.44	10.12	10.28	10.38
168	206	29.5	3.08	10.63	11.07	11.24	11.33
169	206	29.0	2.86	10.71	10.68	10.88	10.94
170	206	29.0	2.82	11.00	10.63	10.76	10.90
171	206	29.0	3.23	10.85	11.13	11.24	11.39
172	206	29.5	3.09	10.87	11.08	11.24	11.34
173	206	29.3	3.13	11.05	11.08	11.24	11.34
174	206	31.0	2.70	10.93	10.99	11.14	11.25
175	206	31.0	3.06	11.19	11.42	11.62	11.69
176	206	30.0	2.93	11.28	11.02	11.13	11.28
177	206	31.0	3.35	12.10	11.77	11.98	12.03
178	206	32.6	3.03	12.18	11.79	11.86	12.05
179	206	32.4	3.03	12.03	11.74	11.86	12.00
180	206	33.8	3.39	12.87	12.52	12.71	12.78
181	206	34.3	3.44	13.09	12.70	12.84	12.97
182	206	35.3	3.40	13.09	12.91	13.08	13.17
183	209	31.0	3.95	12.84	12.49	12.70	12.75
184	209	32.0	3.14	11.23	11.77	11.86	12.03
185	209	32.0	2.59	10.44	11.11	11.26	11.37
186	209	32.0	2.80	10.45	11.36	11.50	11.62
187	209	32.0	2.40	10.20	10.88	11.02	11.14
188	209	30.0	2.64	10.09	10.67	10.77	10.93
189	209	30.0	2.68	10.22	10.72	10.89	10.98
190	209	29.8	2.67	10.41	10.65	10.89	10.92
191	209	29.0	2.54	10.99	10.30	10.40	10.56
192	209	30.3	2.72	10.21	10.84	11.01	11.10
193	209	31.0	2.96	10.63	11.30	11.50	11.57
194	209	29.5	2.72	10.70	10.64	10.76	10.90
195	209	30.5	3.00	10.77	11.23	11.37	11.49
196	209	31.5	3.09	11.39	11.58	11.74	11.85
197	209	28.5	2.96	11.04	10.68	10.87	10.94
198	209	28.6	3.00	10.81	10.75	10.87	11.01
199	209	29.8	3.14	11.18	11.22	11.37	11.48
200	209	29.5	3.35	11.21	11.40	11.60	11.66
201	209	31.0	3.05	11.41	11.41	11.62	11.67
202	209	30.8	2.54	10.81	10.75	10.90	11.01
203	209	31.0	3.24	11.46	11.64	11.74	11.90
204	209	30.0	2.73	10.93	10.78	10.89	11.04
205	209	29.5	2.74	10.62	10.66	10.76	10.92
206	209	30.5	3.00	11.20	11.23	11.37	11.49
207	209	30.0	2.95	10.97	11.04	11.25	11.30
208	209	30.0	3.08	11.44	11.20	11.37	11.46
209	209	31.4	2.72	11.20	11.11	11.26	11.38
210	209	31.0	3.15	11.34	11.53	11.74	11.79
211	209	29.8	2.95	10.87	10.99	11.25	11.25
212	209	30.9	3.08	11.39	11.42	11.62	11.68
213	209	30.7	2.87	11.30	11.12	11.25	11.38
214	209	31.5	2.94	11.53	11.40	11.50	11.67
215	209	31.5	3.06	11.57	11.55	11.74	11.81

TABLE 16.—*Comparative determinations of total solids in milk*—Continued.

Test No.	Cow No.	Specific gravity (Quevenne degrees).	Fat.	Total solids.			
				Gravimetric.	Babcock.	Richmond.	Fleischmann.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
216	209	31.0	3.18	11.59	11.57	11.74	11.83
217	209	31.3	2.85	11.62	11.25	11.50	11.51
218	209	31.3	3.69	12.49	12.25	12.46	12.52
219	209	32.2	3.75	12.68	12.55	12.62	12.81
220	209	33.0	3.32	12.61	12.23	12.35	12.50
221	209	33.3	3.33	12.53	12.32	12.47	12.58
222	209	32.6	3.29	12.28	12.10	12.22	12.36
223	209	33.5	3.27	12.76	12.30	12.47	12.56
224	209	35.6	3.52	13.20	13.12	13.20	13.39
225	209	36.0	3.74	13.70	13.49	13.56	13.75
226	209	34.4	3.75	13.70	13.10	13.25	13.36
227	209	37.0	3.76	13.98	13.76	13.93	14.02
228	209	34.4	4.05	13.56	13.46	13.68	13.72
229	300	33.0	5.19	14.53	14.48	14.63	14.74
230	300	33.3	4.54	13.76	13.77	13.91	14.04
231	300	33.4	4.18	13.76	13.37	13.55	13.63
232	300	33.5	4.49	13.94	13.76	13.91	14.03
233	300	33.4	4.38	13.93	13.61	13.79	13.87
234	300	33.5	4.22	13.51	13.44	13.55	13.70
235	300	33.5	4.46	13.42	13.73	13.91	13.99
236	300	33.1	4.07	13.76	13.16	13.31	13.42
237	300	33.7	4.23	13.53	13.50	13.55	13.76
238	300	33.6	4.22	13.53	13.46	13.55	13.73
239	300	33.5	4.05	13.41	13.24	13.43	13.50
240	300	33.7	4.01	13.20	13.24	13.31	13.50
241	300	33.0	4.16	13.24	13.24	13.43	13.51
242	300	32.8	4.08	13.39	13.10	13.31	13.36
243	300	32.7	4.11	13.44	13.11	13.18	13.37
244	300	32.4	3.98	13.26	12.88	13.06	13.14
245	300	32.6	3.55	12.89	12.41	12.58	12.67
246	300	32.0	4.13	13.42	12.96	13.06	13.22
247	300	31.8	4.29	13.13	13.10	13.30	13.36
248	300	32.0	4.20	12.69	13.04	13.18	13.30
249	300	34.0	4.10	13.04	13.42	13.55	13.68
250	300	33.4	4.12	13.07	13.29	13.43	13.56
251	300	34.0	4.02	13.44	13.32	13.43	13.59
252	300	35.0	4.13	13.40	13.71	13.80	13.97
253	300	35.0	4.22	13.13	13.81	13.92	14.08
254	300	35.0	4.08	13.34	13.65	13.80	13.91
255	300	34.0	4.17	13.76	13.50	13.67	13.77
256	300	33.0	4.32	13.39	13.43	13.55	13.70
257	300	33.0	3.89	12.56	12.92	13.07	13.18
258	300	31.5	3.65	12.57	12.26	12.46	12.52
259	300	33.0	3.22	12.04	12.11	12.23	12.38
260	300	32.0	3.94	12.40	12.73	12.82	12.99
261	300	33.0	3.65	12.35	12.63	12.83	12.89
262	300	32.0	3.61	12.28	12.33	12.46	12.60
263	300	32.5	3.31	12.13	12.10	12.22	12.36
264	300	32.9	3.37	12.28	12.27	12.47	12.53
265	300	33.0	3.45	12.17	12.39	12.59	12.65
266	300	32.0	3.38	11.98	12.06	12.22	12.32
267	300	31.4	3.36	12.07	11.88	12.10	12.15
268	300	30.2	3.50	11.89	11.75	11.85	12.01
269	300	31.2	3.23	11.70	11.68	11.74	11.94
270	300	30.5	3.35	11.57	11.65	11.85	11.91
271	300	30.4	3.15	11.58	11.38	11.61	11.64
272	300	31.3	3.47	11.96	11.99	12.22	12.25
273	300	32.0	3.30	11.70	11.96	12.10	12.22
274	300	31.6	3.35	11.38	11.92	12.10	12.18
275	300	30.1	3.44	11.26	11.65	11.73	11.92
276	300	31.0	3.40	11.38	11.83	11.98	12.09
277	300	30.2	2.98	11.40	11.13	11.25	11.39
278	300	30.2	3.20	11.34	11.39	11.49	11.65
279	301	34.0	4.22	13.38	13.56	13.67	13.83
280	301	33.0	4.15	13.43	13.23	13.43	13.49
281	301	32.3	4.66	13.55	13.67	13.90	13.93
282	301	32.0	4.44	13.43	13.33	13.42	13.59
283	301	33.0	4.29	13.41	13.40	13.55	13.66
284	301	32.4	4.72	13.44	13.76	13.90	14.03
285	301	32.9	4.22	13.36	13.29	13.43	13.55
286	301	32.5	4.37	13.35	13.37	13.54	13.63
287	301	32.8	4.61	13.51	13.73	13.91	14.00
288	301	31.5	4.72	13.78	13.54	13.66	13.80
289	301	34.2	4.16	12.98	13.54	13.67	13.81
290	301	33.2	4.40	13.33	13.58	13.67	13.84
291	301	32.2	4.31	13.54	13.22	13.30	13.49
292	301	33.0	4.42	13.67	13.55	13.67	13.82

TABLE 16.—*Comparative determinations of total solids in milk*—Continued.

Test No.	Cow No.	Specific gravity (Quevenne degrees).	Fat.	Total solids.			
				Gravimetric.	Babcock.	Richmond.	Fleischmann.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
293	301	33.7	4.02	13.47	13.25	13.31	13.51
294	301	33.5	3.88	13.41	13.03	13.19	13.29
295	301	34.2	3.92	13.27	13.25	13.31	13.52
296	301	34.0	4.09	13.99	13.41	13.55	13.67
297	301	34.0	4.24	13.31	13.59	13.67	13.85
298	301	33.5	4.20	12.83	13.42	13.55	13.68
299	301	33.0	4.34	13.37	13.46	13.55	13.72
300	301	33.0	3.72	12.91	12.71	12.83	12.98
301	301	33.0	4.23	13.46	13.33	13.43	13.59
302	301	34.0	4.25	13.36	13.60	13.79	13.86
303	301	33.0	4.32	13.23	13.43	13.55	13.70
304	301	35.5	4.11	13.71	13.81	13.92	14.07
305	301	34.0	3.89	13.34	13.17	13.31	13.43
306	301	34.0	3.56	12.94	12.77	12.95	13.04
307	301	33.5	3.90	12.80	13.06	13.19	13.32
308	301	34.0	3.98	13.02	13.28	13.43	13.54
309	301	31.7	3.58	12.54	12.22	12.34	12.46
310	301	31.1	3.69	12.37	12.20	12.34	12.47
311	301	33.8	3.71	12.05	12.90	13.07	13.17
312	301	32.0	3.75	12.36	12.50	12.70	12.76
313	301	33.0	3.83	12.61	12.85	12.95	13.11
314	301	32.5	3.80	12.64	12.69	12.82	12.96
315	301	33.0	3.85	12.66	12.87	13.07	13.13
316	301	32.0	3.71	12.74	12.45	12.58	12.72
317	301	32.5	3.75	12.67	12.63	12.82	12.89
318	301	32.0	4.01	12.80	12.81	12.94	13.08
319	301	33.0	3.75	12.53	12.75	12.95	13.01
320	301	32.5	4.12	13.11	13.07	13.18	13.33
321	301	32.6	3.86	12.93	12.78	12.94	13.06
322	301	33.6	4.24	13.31	13.49	13.55	13.75
323	301	32.4	4.09	13.01	13.01	13.18	13.27
324	301	32.8	4.00	12.74	13.00	13.19	13.26
325	301	33.6	3.71	13.01	12.85	12.95	13.12
326	301	32.9	3.65	12.77	12.61	12.83	12.87
327	301	33.0	3.76	12.90	12.76	12.95	13.08
328	301	32.6	4.07	12.90	13.03	13.18	13.30
329	301	33.0	3.77	12.50	12.77	12.95	13.04
330	301	32.1	4.00	12.26	12.83	12.94	13.09
331	301	32.0	3.96	12.85	12.75	12.94	13.02
332	301	31.2	4.16	12.38	12.79	12.94	13 06
333	301	31.4	3.76	12.71	12.36	12.58	12.63
334	301	32.7	3.76	12.67	12.69	12.82	12.95
335	301	32.5	4.07	12.79	13.01	13.18	13.27
336	301	32.9	3.55	12.86	12.49	12.71	12.75
337	301	31.5	3.84	12.16	12.48	12.58	12.75
338	301	31.3	3.61	12.09	12.16	12.34	12.42
339	301	32.5	3.67	12.84	12.53	12.70	12.79
340	301	31.2	3.71	12.34	12.25	12.34	12.52
341	302	31.8	4.88	13.98	13.81	14.02	14.07
342	302	31.0	4.73	13.70	13.43	13.54	13.69
343	302	32.0	3.92	12.80	12.70	12.82	12.97
344	302	31.6	4.08	12.88	12.80	12.94	13.06
345	302	32.0	4.47	12.97	13.36	13.54	13.63
346	302	32.9	4.11	12.52	13.16	13.31	13.42
347	302	32.8	4.48	13.45	13.58	13.79	13.84
348	302	31.8	4.48	13.31	13.38	13.54	13.59
349	302	31.6	4.71	13.67	13.55	13.66	13.82
350	302	32.8	4.07	12.87	13.08	13.31	13.35
351	302	31.0	4.71	13.15	13.40	13.54	13.67
352	302	29.5	5.18	13.54	13.59	13.76	13.85
353	400	34.5	4.48	14.16	14.00	14.16	14.26
354	400	35.5	4.07	13.83	13.76	13.92	14.02
355	400	36.0	3.88	13.51	13.66	13.80	13.92
356	400	36.0	4.03	13.35	13.84	13.92	14.10
357	400	34.0	4.33	13.58	13.70	13.79	13.96
358	400	31.3	3.95	13.25	12.57	12.82	12.83
359	400	33.7	3.92	12.98	13.13	13.19	13.39
360	400	33.6	4.17	13.35	13.40	13.55	13.67
361	400	33.0	4.13	13.58	13.21	13.31	13.47
362	400	32.5	4.31	13.23	13.30	13.42	13.56
363	400	33.5	3.99	12.87	13.16	13.31	13.43
364	400	34.0	3.54	12.52	12.75	12.83	13.01
365	400	32.0	3.80	12.05	11.96	12.10	12.22
366	400	33.0	3.99	12.81	13.04	13.19	13.30
367	400	34.0	3.57	12.11	12.78	12.95	13.05
368	400	32.8	3.73	12.96	12.68	12.83	12.94
369	400	33.1	3.53	12.91	12.51	12.71	12.78

TABLE 16.—*Comparative determinations of total solids in milk*—Continued.

Test No.	Cow No.	Specific gravity (Quevenne degrees).	Fat.	Total solids.			
				Gravimetric.	Babcock.	Richmond.	Fleischmann.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
370	400	35.1	3.92	13.28	13.48	13.56	13.74
371	400	34.0	3.78	12.94	13.04	13.19	13.30
372	400	35.0	3.55	13.11	13.01	13.20	13.27
373	400	34.6	3.62	13.17	12.99	13.08	13.26
374	400	33.4	3.72	13.01	12.81	12.95	13.08
375	400	33.7	3.61	13.01	12.76	12.83	13.02
376	400	34.3	3.79	13.00	13.12	13.32	13.39
377	400	35.0	3.52	12.69	12.97	13.08	13.24
378	400	34.0	4.07	12.99	13.38	13.55	13.65
379	400	34.0	3.85	13.18	13.12	13.31	13.38
380	400	33.2	3.95	12.97	13.04	13.19	13.30
381	400	33.7	3.53	12.88	12.66	12.71	12.92
382	400	34.4	3.90	13.26	13.28	13.44	13.54
383	400	34.6	3.96	13.34	13.40	13.56	13.66
384	400	34.7	3.53	12.85	12.94	12.96	13.17
385	400	32.6	3.43	12.40	12.27	12.34	12.53
386	400	32.0	3.91	13.03	12.69	12.82	12.96
387	400	34.0	4.52	13.48	13.92	14.03	14.19
388	402	36.0	4.49	14.06	14.39	14.52	14.65
389	402	35.0	5.14	14.32	14.92	15.00	15.18
390	402	32.9	4.48	13.62	13.60	13.79	13.87
391	402	32.6	4.08	12.94	13.05	13.18	13.31
392	402	32.8	4.31	13.18	13.37	13.55	13.64
393	402	33.0	4.20	13.34	13.29	13.43	13.55
394	402	34.5	4.20	13.41	13.67	13.80	13.93
395	402	34.0	3.96	13.23	13.25	13.43	13.52
396	402	34.0	4.21	13.46	13.55	13.67	13.82
397	402	33.5	3.92	12.91	13.08	13.19	13.34
398	402	32.5	3.77	12.83	12.65	12.82	12.91
399	402	34.0	3.99	13.07	13.29	13.43	13.55
400	402	33.7	3.61	12.78	12.76	12.83	13.02
401	402	34.0	4.03	13.41	13.34	13.43	13.60
402	402	34.4	3.69	13.01	13.03	13.20	13.29
403	402	34.0	3.86	13.06	13.13	13.31	13.40
404	402	33.0	3.92	13.06	12.95	13.07	13.22
405	402	34.0	3.84	13.26	13.11	13.19	13.37
406	402	34.4	3.80	13.21	13.16	13.32	13.42
407	402	33.4	3.80	13.11	12.91	13.07	13.17
408	402	33.7	3.79	13.15	12.97	13.07	13.24
409	402	34.0	3.93	12.94	13.22	13.31	13.48
410	402	34.4	3.78	12.86	13.14	13.32	13.40
411	402	33.6	4.16	12.94	13.39	13.55	13.66
412	402	32.7	3.94	13.10	12.90	12.94	13.17
413	402	32.7	4.12	12.69	13.12	13.18	13.38
414	402	33.4	3.76	13.02	12.86	13.07	13.13
415	402	34.5	4.09	13.26	13.53	13.68	13.80
416	402	34.0	4.20	13.46	13.54	13.67	13.80
417	403	35.8	4.06	13.63	13.82	14.04	14.08
418	403	36.0	3.53	12.85	13.24	13.32	13.50
419	403	33.8	3.45	12.42	12.59	12.83	12.85
420	403	33.4	3.27	12.49	12.27	12.47	12.54
421	403	33.3	3.35	12.21	12.35	12.59	12.61
422	403	32.7	3.35	12.07	12.20	12.34	12.46
423	403	33.3	3.45	12.12	12.47	12.71	12.73
424	403	32.0	3.36	12.22	12.03	12.22	12.30
425	403	32.0	3.33	11.61	12.00	12.10	12.26
426	403	32.3	2.96	11.86	11.63	11.86	11.89
427	403	32.9	3.16	11.53	12.02	12.23	11.28
428	403	33.6	3.26	12.00	12.31	12.47	12.58
429	403	32.3	3.01	11.32	11.69	11.86	11.95
430	403	31.6	3.19	11.40	11.73	11.86	11.99

NOTE.—Cows Nos. 4, 99, and 118 are Jerseys; Nos. 205, 206, and 209 are Holstein-Friesians; Nos. 300, 301, and 302 are Ayrshires, and Nos. 400, 402, and 403 are Shorthorns.

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A COMPARATIVE STUDY OF METHODS OF EXAMINING FECES FOR EVIDENCES OF PARASITISM.

BY

MAURICE C. HALL,
Assistant Zoologist, Zoological Division.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

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LETTER OF TRANSMITTAL.

UNITED STATES DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., January 17, 1911.

SIR: I have the honor to transmit the accompanying manuscript of an article entitled "A Comparative Study of Methods of Examining Feces for Evidences of Parasitism," by Mr. Maurice C. Hall, Assistant Zoologist in the Zoological Division of this bureau, with the recommendation that it be published as a bulletin of this bureau.

Mr. Hall has devised a method of examining feces by means of which the presence or absence of parasites infesting the alimentary tract may be rapidly and accurately determined. Various methods of fecal examination are in vogue among medical and zoological investigators, and these are all critically compared with Mr. Hall's method, which is shown to yield superior results.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

Page
Introduction 7
Methods of microscopic examination 7
  Smear method 7
  Sedimentation method 8
  Burette method 8
  Centrifuge methods 8
  Sieve methods 8
  Gasteiger's filter method 9
  Bass's salt solution method 9
  Bass's calcium chlorid centrifuge method 10
  Garrison's calcium chlorid sedimentation method 10
  Wellman's sodium acetate centrifuge method 11
  Pepper's adhesion method 11
  Telemann's ether hydrochloric acid method 11
  Culture methods 12
Purpose of methods 12
  Advantages of concentration 12
  Properties used in concentration 13
    Physical properties 13
    Chemical properties 14
    Biological properties 14
  The theoretical ideal 15
  Practical objections 15
Gross examination 15
The writer's method 16
  Comminution of feces 16
  Sieving 18
  Sedimenting and centrifuging 21
  Preparation of slides 21
  Concentration obtained by the use of sieves 22
  Summary of method 22
  Collection index 23
Tests of unpublished methods 24
Comparative tests of the writer's method and other methods 25
  Smear method 25
  Sedimentation method 25
  Burette method 25
  Gasteiger's filter method 26
  Bass's calcium chlorid centrifuge method 26
  Garrison's calcium chlorid sedimentation method 28
  Telemann's ether hydrochloric acid method 28
  Culture methods 30
Objections to certain methods 31
  Injury to parasites 31
  Limited application or incommensurate results 32
Disinfection of feces 32
Summary 33
  Adaptability of method 33
  Economy of method 34
  Conclusion 34
Bibliography 35

ILLUSTRATION.

Page
FIG. 1. Apparatus used in the writer's method of examining feces............ 17

A COMPARATIVE STUDY OF METHODS OF EXAMINING FECES FOR EVIDENCES OF PARASITISM.

INTRODUCTION.

Having occasion to make numerous examinations of feces for evidences of parasitic infection, the writer has devoted some time to developing a satisfactory method which would be capable of general application in routine examinations and which would give the surest results in the shortest time, one which would detect any existing infection, even though light, in any kind of feces, so far as the existing limitations of fecal examinations permit. The different methods which have been used and advocated, most of them being methods used in examining human feces, were tested on feces of various kinds. This paper deals with the results of these tests. The method which gave the most satisfactory results and which is hereafter referred to as the writer's method is a modification of previous methods.

Fecal examinations are of two sorts—first, examination with the naked eye for gross evidence of infection in the shape of entire parasites or fragments, and, second, microscopic examination for parasite eggs, embryos, etc. The first is very commonly, perhaps wrongly, neglected; the second is commonly used and will be considered first here.

METHODS OF MICROSCOPIC EXAMINATION.

SMEAR METHOD.

The microscopic method which is commonly used and advocated is what may be called the smear method. A small particle of feces is taken up with a toothpick or stirring rod, smeared on a glass slide in a drop of water or salt solution, covered with a cover glass, and examined under the miscroscope. This method is the oldest and the simplest, can be used under almost any conditions, and, in that it calls for no equipment beyond a toothpick, a slide and cover glass, and a microscope, is also the most inexpensive. It is usually considered satisfactory, providing a number of slide preparations, commonly 10, are examined. This method has been repeatedly commended by

Stiles (1902hh, 1903l, 1906a), is one commonly used by physicians (Jones, 1907, et al.), and was used by Garrison, Ransom, and Stevenson (1903a), and Stiles and Garrison (1906a) in Washington, and by Garrison (1908) in Manila.

SEDIMENTATION METHOD.

The simplest modification of this method consists in allowing the feces, if sufficiently fluid, to settle, and then examining the sediment; if the feces are too solid for this, large or small amounts are washed in a sufficient quantity of water, decanted as long as any matter will float, and the sediment finally examined. Stiles, in the papers just cited, Braun (Braun and Lühe, 1909), Garrison (1910), Letulle (1905), Jones (1907), and others have advocated this sedimentation method.

BURETTE METHOD.

I am informed by Dr. Ransom that one writer, in an article to which I have not a reference at present, has varied the preceding method by taking the sediment from the bottom, through a stopcock, a method designated in this paper as the burette method.

CENTRIFUGE METHODS.

Another variation of the sedimentation method is to use a centrifuge for the purpose of giving a more rapid and certain concentration of material. Pepper (1908) states that he has found repeated centrifuging, in which the sediment is shaken up each time with some fresh water, very useful. This is the process commonly known as washing. Bass (1909) uses the same device as part of his method. Braun (Braun and Lühe, 1909) notes that under certain circumstances the sediment may be centrifuged after sedimenting and decanting. He does not state under what circumstances this is to be done. Letulle (1905) admits the use of the centrifuge if needed, but objects to it on the ground that certain eggs suffer mechanical injury from its use. Stiles (1902hh, 1903l) states: "The centrifuge does not appear to be of any special value in fecal examinations."

SIEVE METHODS.

Another variation of technique consists in the use of one or more sieves or screens to take out coarse particles of undigested food and similar objects. Stiles (1902hh, 1903l) gives a qualified indorsement of this method in the following terms:

> In case an unusually large amount of large, coarse material is present in the feces, it is sometimes convenient to pour the entire mass through a sieve, re-

jecting the portion left in the sieve; or to wash the feces in a sieve, holding the latter under water. As a rule, however, the sieve is not very useful in fecal examinations.

On the other hand, the use of the sieve receives unqualified indorsement from Cobb (1904). In examining sheep feces for fluke eggs he boils a pellet or part of a pellet of sheep dung in water for a few minutes; puts it in a hemispherical brass sieve of 80 to 100 meshes to the inch, the sieve being in a watch glass full of water, and itself standing partly full of water; brushes the pellet through the sieve by means of a sable brush; sediments the fluid containing the fecal matter which passes through the sieve, and repeatedly removes the supernatant muddy fluid with a pipette until water poured on the sediment remains clear; and then brushes this sediment through a sieve of miller's silk bolting cloth of five meshes to the millimeter. The water which passes through the silk sieve is then examined for fluke eggs.

Bass (1909), Telemann (1908), and Garrison (1910) use a sieve of some sort in making examinations of feces.

GASTEIGER'S FILTER METHOD.

Gasteiger's (1904) method is an inversion of the methods where a centrifuge and sieve are used. Whereas with the latter the object is to screen out foreign particles larger than parasites and eggs and seek for the parasitic material at the bottom of the centrifuge tube, Gasteiger, in his search for eggs of *Ascaris* in the stalls of cattle, soaks the manure, straw, and mud in water; filters this water through some unspecified sort of filter, and examines the residue, not the filtrate, for eggs. This accomplishes to a lesser degree the same thing that is accomplished by sedimentation, sieving, and centrifuging—it gives a concentration of material.

BASS'S SALT SOLUTION METHOD.

Bass (1906) proposed a method which consisted in putting a quantity of feces in a vial three-fourths full of a nine-tenths saturated solution of sodium chlorid, shaking this well, allowing it to settle, and then examining a drop from the surface of the fluid. This method was proposed for the examination of human feces for hookworm eggs. According to Bass, all the eggs rise to the surface and large numbers may be found in one drop. Of course this method depends on the specific gravity of the parasite egg being less than that of the solution employed. Waddell (1910) states that if an examination of three slides, presumably smear preparations, did not disclose human hookworm eggs, this method of Bass's was used, and says: "By this method from 30 to 40 minutes could be saved per stool examined."

BASS'S CALCIUM CHLORID CENTRIFUGE METHOD.

Afterwards Bass (1909) modified the above method as follows: A quantity of feces is diluted with water, 1 in 10, and strained through gauze to get rid of coarse particles. What comes through is centrifuged, the fluid poured off, the centrifuge tube refilled and the fresh material and the old sediment centrifuged again, thereby constantly adding to the total sediment, until all the diluted feces have been used. The sediment is rewashed several times until all matter that can be washed out in this manner is removed. Then a calcium chlorid solution of a specific gravity of 1.050 is substituted for the water. This disposes of everything having a specific gravity below 1.050, and the sediment may be examined at this point. If much sediment remains, the heavier matter may be removed by centrifuging with a calcium chlorid solution having a specific gravity of 1.250. In this solution the eggs come to the top and a few drops from the surface may be removed and examined, or, better, some of the top fluid may be poured off, diluted with water sufficiently to bring the specific gravity below 1.050, and centrifuged. The sediment will now contain most of the eggs that were in the original amount of feces and may all be put on a slide and examined.

GARRISON'S CALCIUM CHLORID SEDIMENTATION METHOD.

Garrison (1910) has outlined a method which is essentially a modification of Bass's (1909) method. According to Garrison—

> The specific gravity of the ova is from 1.050 to 1.100 (old eggs sometimes higher, according to Bass). If the stool be liberally diluted with tap water the mixture has a considerably lower specific gravity, which varies, of course, with the character of the stool, but is usually about 1.005, so that the ova, together with the heavier sediment, sink to the bottom. [Allow to stand for an hour or more; decant and add fresh water repeatedly until soluble matter is washed out.] At any time during the sedimentation, but preferably after the specimen has been washed a few times, the coarser material may be removed by straining and washing the sediment through a fine wire gauze, using a small, strong jet of water. To completely wash a specimen until the supernatant water is clear may require quite a number of sedimentations, and it may be desirable to continue the process throughout one or more days. * * * Sometimes the feces contain heavy, gritty, solid material, which is particularly annoying in making slide preparations. The specific gravity of much of this material is sufficiently higher than that of the eggs to allow the use of a solution with a specific gravity between the two which will float the eggs to the surface and allow the heavy sediment to sink. This may be done by suspending the specimen, preferably after it has been well washed, in a solution of calcium chlorid containing 350 grams to the liter of water, which gives a specific gravity of about 1.200. (A saturated solution of the commercial salt has about the same specific gravity.) The top layer, containing eggs and the lighter débris, is decanted, leaving the heavy sediment behind.

WELLMAN'S SODIUM ACETATE CENTRIFUGE METHOD.

Wellman (1910) employs a method which appears to be a modification of Bass's (1909) method, but as it is not clear just what Wellman means by his statement of his method, the writer has not tested it. His method, which is for hookworm eggs, is given as follows:

> The principle involved was first employed independently by the writer and by Dr. Bass, of New Orleans, Dr. Bass being the first to publish his results. Our own method is to use two solutions of sodium acetate of specific gravities of 1.050 and 1.250, respectively. A portion of suspected feces is mixed with one of these solutions and centrifuged for about 10 seconds, the liquid decanted, then the other solution is poured on and mixed gently, the whole again centrifuged, and this process repeated until the eggs are all in one layer, the sand and other heavy ingredients of the feces remaining below and the light flocculent components lying above the zone in which the eggs remain. With this technique one slide contains as many ova as 50 or 100 slides by the ordinary method. Dr. Bass uses calcium chlorid for his solutions, but the writer has not been able to employ this salt satisfactorily on account of its hygroscopic properties.

It is unfortunate that Wellman does not state just why the hygroscopic properties of calcium chlorid are objectionable, as Bass employs this salt on account of these properties.

PEPPER'S ADHESION METHOD.

An interesting application of technique to a particular case is that of Pepper (1908). He takes advantage of the stickiness which he finds to be a property of human hookworm eggs in the following manner: Washed and sedimented feces are put on a slide for a few minutes and then immersed gently in water; on lifting it out the eggs are found adhering after everything else has been washed away. This process may be repeated and numerous eggs collected. Pepper does not find the same stickiness in the case of *Ascaris*, *Trichuris*, or *Tænia* eggs.

Stiles (1910) has given the smear method and the methods of Pepper (1908) and Bass (1909) for the examination of human feces for evidence of hookworm infection.

TELEMANN'S ETHER HYDROCHLORIC ACID METHOD.

Another modification of method consists in using chemical as well as physical means to secure a concentration of parasitic material. Telemann (1908), who appears to have been the first to use chemical methods in examining feces for parasite eggs, uses the following technique: Small particles are taken from a number of places in the feces under investigation and shaken up in a mixture of equal parts of ether and pure hydrochloric acid in a reagent glass. (I believe that I have had slightly better results from adding ether first, stirring well, and then adding the hydrochloric acid.) The ether dissolves the neutral fats and free fatty acids, while the hydrochloric acid dissolves the albuminous matter, as casein, etc., soaps, mucin, phosphates, and various calcium salts. This mixture is then filtered through a fine hair sieve to eliminate large particles, and the liquid is then centrifuged about a minute. In the centrifuge tube will be found three layers, an upper one of fats dissolved in ether, a middle one of bacteria and small detritus in the acid, and finally a sediment of small food particles, mostly cellulose and muscle fibers, and parasite eggs. The parasite eggs, being relatively more concentrated in the sediment than in the original feces, can readily be found in the usual manner by microscopic examination of a slide. This method has been tested and commended by Quadflieg (1909), who notes, however, that it should be supplemented by the smear method, as the latter seems to be superior in the case of certain parasites. Pfister (1909) commends the method as being satisfactory in searching for *Bilharzia* eggs.

CULTURE METHODS.

A final modification of technique is to use culture methods, the feces being kept moist and warm, and after a proper interval being examined for embryos which have developed and escaped from the eggs. Under certain conditions this method gives a concentration of material on the glass receptacle or in water contained in depressions on the surface of the feces.

PURPOSE OF METHODS.

Inasmuch as the writer's method is a variation of some existing methods already outlined, it may be in order at this point to find a basis for the selection of a method by considering what the purpose of the microscopic examination and the reason for any method may be.

Obviously, the purpose of the examination is to detect parasites and their eggs in the feces if they are present. The evident way to accomplish this is to examine the feces on a microscopic slide preparation after they are so thinned as to permit of examination. Every method, no matter how complicated, includes these essential steps. The smear method accomplishes this in the minimum number of steps: fluid feces are examined at first hand; solid feces are rendered fluid and then examined or are mounted in a drop of water.

ADVANTAGES OF CONCENTRATION.

The purpose of all modifications of this simple proceeding, as the preceding sketch shows, is to attain one object—the concentration of parasites and their eggs in order to facilitate the detection of infection. This concentration is accomplished by increasing the time spent on the treatment of the feces previous to their microscopic examination. At the same time it increases the likelihood of finding parasites and their eggs when these are present.

The justification for thus shifting the greater amount of time from that of microscopic examination to that of preliminary treatment is readily found. Microscopic examination of feces for parasites and their eggs is one which calls for some degree of mental concentration. The judgment is constantly exercised in passing upon various objects which catch the eye and by a superficial resemblance in size, shape, color or refraction to parasites and eggs call for a determination of their spurious character. The determination of some of these forms requires the use of the higher powers of the microscope and some little time in examination. In addition to the fact that microscopic examination makes considerable mental demands, is the fact that microscopic work is eyestrain—a matter of considerable importance to anyone who has much work of the kind to do—and the saving of eyestrain by eliminating part of the objects which must be picked up by the eye, only to be rejected as not being the thing sought for, is greatly to be desired.

On the other hand, the treatment of feces preliminary to microscopic examination calls for little mental concentration and no eyestrain, and so leaves the mind free for other things and prolongs the period of usefulness of the eyes. Moreover, the fact that this preliminary treatment can largely be left to the operation of natural laws and to power machinery also saves to the operator the time when things can be left unattended.

PROPERTIES USED IN CONCENTRATION.

Concentration, the object of all improvements on the smear method, is obtained by eliminating as much as possible of the nonparasitic material by taking advantage of differences in physical, chemical, and biological properties between such material and the parasites. These differences are in most cases those of specific gravity, of size, of physical and chemical solubility, of adhesiveness, and of capacity for growth and development.

PHYSICAL PROPERTIES.

The elimination of matter having a specific gravity less than that of the parasites is accomplished by sedimenting in water and decanting material which floats or remains suspended, and by centrifuging in water, and is based on the general truth that parasites are heavier than water and will settle to the bottom. Dock and Bass (1910) lay emphasis on the fact that—

> Most * * * food particles are irregular in outline and shape, making their surface per given weight greater than that of the round, oval eggs.

Owing to the greater resistance offered by such surfaces, these particles are slower in reaching the bottom.

In Bass's (1909) second method additional matter of specific gravity less than that of the parasite is eliminated by the use of a calcium-chlorid solution having a specific gravity of 1.050. In this method and in Bass's (1906) first method matter having a specific gravity greater than that of the parasites is also eliminated, in the first method by the use of a nine-tenths saturated solution of sodium chlorid and in the second method by the use of a calcium-chlorid solution having a specific gravity of 1.250. Garrison (1910) varies this method by using only one solution, of specific gravity 1.200, and Wellman substitutes sodium acetate for calcium chlorid.

These methods and the sedimentation and filtration methods likewise eliminate matter which is soluble in water. Washing not only assists in concentrating material by removing fine suspended and colored soluble matter, but in so doing it gives a clearer background for microscopic work.

The elimination of matter larger than the parasites is accomplished by the use of sieves with a mesh aperture of such size that it will permit the passage of parasitic material, but not of coarse fecal particles.

The elimination of matter having different adhesive properties, as advocated by Pepper (1908), has only a limited application and needs no discussion in a consideration of general methods. The writer has tested this method and finds it very satisfactory for the purpose for which it was proposed. Dock and Bass (1910) commend Pepper's method, and state in comment:

> The best results are obtained if a part of the fecal material is removed by use of the centrifugal. Otherwise the method is often disappointing.

Other methods of differentiation, as staining reaction, etc., might be devised. In fact, as later indicated, the writer has attempted something of the sort without obtaining satisfactory results. Nevertheless satisfactory methods might be devised.

CHEMICAL PROPERTIES.

The elimination of matter having a different chemical solubility is accomplished by the addition of chemicals which will dissolve part of the nonparasitic substances with the formation of new and soluble substances. Such chemicals will also dissolve a number of things forming a mere physical solution.

BIOLOGICAL PROPERTIES.

The elimination of the nonparasitic matter lacking the biological properties of growth and development is accomplished by the use of culture methods. These methods, which are indispensable in bacteriology and of increasing importance in protozoology, in both of which the reproductive faculty may be taken advantage of, are of limited use in the examination of feces for worm parasites, as multiplication of the parasites under these circumstances is only possible in such exceptional cases as *Strongyloides*.

THE THEORETICAL IDEAL.

From the foregoing it would appear that the best technique should eliminate matter of specific gravity different from that of the parasites by sedimentation, or, since it is more rapid, by centrifuging, in solutions of such strengths as to get rid of the lighter nonparasitic material at one time and the heavier at another; such technique should eliminate matter of different size by sieving; finally it should, in the course of the above processes or the filtration method, eliminate matter of physical or chemical solubility different from that of the parasites by the use of suitable reagents. Such a method would be expected to give a slide with the maximum amount of parasitic material and the minimum amount of other material. Incidentally, this treatment would comminute the resultant sediment so that it would give a clear, uniform microscopic field, superior to that obtained by the smear method. An ordinary smear of feces, subjected to no preliminary treatment, makes a very trashy and indifferent microscopic preparation, especially in the herbivora, where large plant particles are easily capable of concealing the evidences of parasitic infection, and the same objection holds good to some extent of smear preparations of any sort of feces.

PRACTICAL OBJECTIONS.

In actual practice, experience modifies the view that we should do all the above things and shows that there are other things that must be considered. Among these are (1) the possible injury to parasites or eggs by certain forms of technique, and (2) the possibility that in general work certain methods may prove to have only a limited application, or the concentration resulting from the application of certain methods may be too insignificant to warrant the time and effort expended. A discussion of these points can best be given after consideration of the writer's method.

GROSS EXAMINATION.

The naked-eye examination of feces for gross evidences of parasites, as stated at the beginning of this paper, receives too little attention, and it may be fairly assumed from the lack of statements showing a definite technique that it usually consists of perhaps a hasty glance at bottled material or possibly some picking over of the feces with a dissecting needle or a stirring rod at the most.

The likelihood of finding easily identified mature worms or tapeworm segments warrants a more careful examination. Garrison (1910) has recognized this fact and given a method of collection as follows:

> In the case of the larger worms, such as *Ascaris* and the large tapeworms, which are easily picked out, about the only precautions needed are to clean the worms of the fecal débris and to keep them as fresh as possible until they are killed. This is best done by transferring them promptly from the fresh stool to a dish of warm physiological salt solution. * * *
>
> Smaller worms, like hookworms, pinworms, whipworms, and the "dwarf tapeworm," require careful searching, and this is best done by spreading portions of the fluid stool in a thin translucent layer in a glass dish or on a glass plate (table top), the glass being placed over a black background.
>
> In picking out the still smaller forms, such as the adult trichinæ, the minute intestinal fluke, *Heterophyes*, and the detached heads of tapeworms, a hand lens is almost necessary, and it is advisable to make a routine practice of running over the material with a magnifier of low power in every case.
>
> Should the stool not be sufficiently fluid to admit of the above manipulation, it must be thoroughly mixed and diluted with water, and this brings up the method of sedimentation, which, while of special use in collecting ova, is frequently a valuable help in finding the smaller worms.

THE WRITER'S METHOD.

The writer's method includes both macroscopic and microscopic examination. Concentration is attained by careful comminution of feces, the use of sieves, sedimentation and centrifuging, and washing in water. The result is checked by centrifuging one tube of material in a calcium chlorid solution with a specific gravity of 1.250.

The illustration (fig. 1) is intended to show at a glance the apparatus needed (except for pipettes and brushes) and the method and order of its use. It is, of course, evident that the reader will be familiar with the individual pieces of apparatus.

COMMINUTION OF FECES.

In any method which aims at a concentration of the parasitic material present it is first necessary to break up the fecal masses in order to release the embedded and adherent parasites and to put the feces in such shape that they can be treated by any of the processes outlined in the preceding part of the paper. This is a feature to which it seems that too little attention is paid. Of the methods of comminuting such fecal masses, boiling in water, as advocated by Cobb (1904) for sheep feces, is fairly good, but not so effective for sheep feces as the general method used by the writer, and has obvious objections in the cases of human feces or those of the carnivora. Stiles (1902hh, 1903l) states that the feces should be shaken or stirred thoroughly, and Telemann (1908) notes that the feces

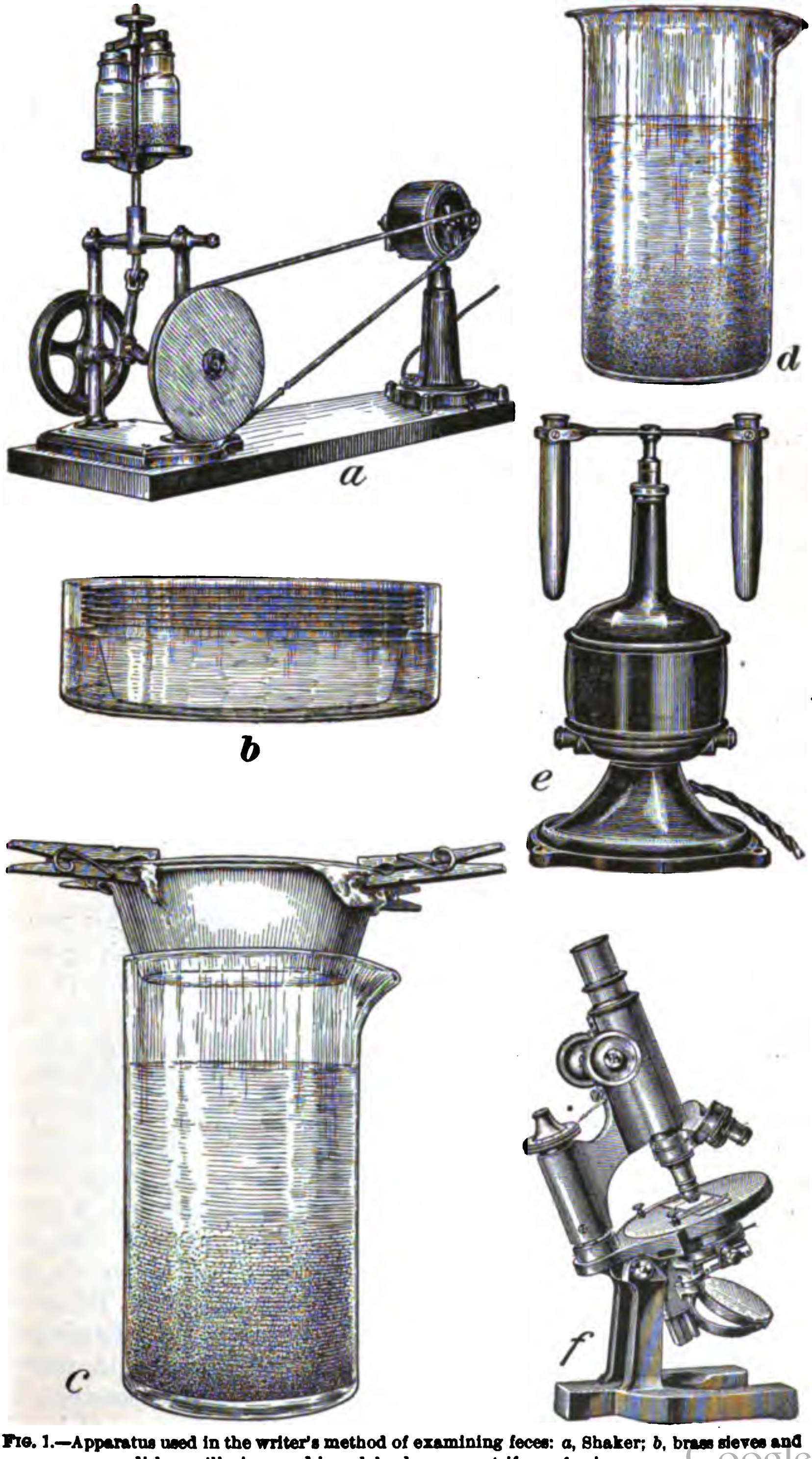

Fig. 1.—Apparatus used in the writer's method of examining feces: *a*, Shaker; *b*, brass sieves and dish; *c*, silk sieve and jar; *d*, beaker; *e*, centrifuge; *f*, microscope.

should be shaken in the mixture of ether and hydrochloric acid. Garrison (1910) advises the use of large quantities of water, 5 to 10 liters, for the first sedimentation of large solid stools, the water to be run in violently and the mixture well stirred.

The writer takes particular care to break up the feces thoroughly. The method consists in shaking the feces in a rubber-stoppered, wide-mouthed glass bottle about three-fourths full of water. The entire fecal sample, up to 4 or 5 ounces, is used. It is sometimes desirable to break or crush with a stirring rod such hard fecal masses as sheep feces. It is also sometimes desirable to add shot to hard fecal masses. In such cases the most satisfactory results were obtained with about 100 lead shot having a diameter of 3.8 millimeters; shot with a diameter of 8 millimeters was not as effective in breaking up feces, and had the additional disadvantage that it blackened the glass. The use of shot is to be avoided as a rule, for the reason that gross parasitic material is apt to be damaged by it. At first the bottle containing the feces and water and, if necessary, the shot, was shaken rapidly by hand, but as the amount of fecal examination in this laboratory warranted the use of a machine for this work, a shaker of the kind used in mixing "milk shakes" was installed and connected by belting with a pulley wheel fitted on an electric-fan motor. This apparatus (fig. 1, *a*) is very rapid and effective in its work. The bottles are lifted a distance of 5 centimeters and dropped back again at a rate of about 500 times per minute. The same machine operated by hand would doubtless be very good.

SIEVING.

After having been broken up in this manner the feces are poured through a set of six brass sieves. The sieves have a mesh aperture ranging from 3 millimeters in the largest to about one-fourth of a millimeter in the smallest. They are made by taking tin pans with a bottom diameter of about 6½ inches and sides 2 inches high, cutting out the bottom, leaving a flange near the sides, and soldering onto the flange brass screening with meshes of various sizes. These sieves are copied from a set used by Dr. Cobb in collecting free-living nematodes. The pans, of course, tend to rust, as it is not always convenient to dry them after use. Dr. Cobb tells me that he avoids this by the use of oil or grease warmed on the pans and then carefully wiped off. As this practice seems inconvenient also, the pans used by the writer have been enameled to prevent rusting. This makes the pans bind a little when nested. A set coated with shellac is being tried. Galvanized-iron, brass, or aluminum pans would presumably be better, though the two latter would be more expensive. The brass sieves which can be purchased already made are not beveled and hence can not be nested. They can be superimposed, one on another, but the result is a stack which is so high that it does not permit of six of them being set in a shallow dish of water with the water standing above the screen in the upper sieve.

The sieves are nested in the order of mesh aperture, with the coarsest on top, and placed in a large porcelain evaporating dish, or a large glass crystallization dish (fig. 1, *b*). The feces are poured into the top screen and pass through the screens to the evaporating dish, particles of different sizes being held by the different screens. The use of fewer screens would not be a gain, as too coarse material poured on a screen clogs it. Tap water or normal salt solution is poured in the upper sieve until the water stands in the evaporating or crystallization dish at a level above that of the bottom of the upper sieve. This sieve is lifted and shaken a little until the fine matter has passed through. It is then lifted out and put in a large crystallization dish half full of water or salt solution and the matter it contained examined on the screen or washed into the dish. Gross parasitic material is picked out, the screen rinsed, the dish emptied and refilled if the amount of discoloration or trash present warrants it, and the process is repeated with the remaining sieves. The gross material left on the screens is thoroughly cleaned, and the likelihood of wasting time examining citrus pulp vesicles, vegetable fibers, etc., as possible parasites is reduced to a minimum.

The sediment left in the evaporating dish after removing the finest sieve is poured onto a screen of miller's silk bolting cloth with a mesh aperture of 0.117 to 0.134 millimeter and the finer particles washed through into a tall jar (fig. 1, *c*). The mesh aperture of this sieve diminishes as the cloth shrinks with use, and in a cloth which has been in use for several months it has diminished to a size of 0.070 to 0.080 millimeter. Such a mesh is too fine to permit the passage of the eggs of such important species as *Fasciola hepatica*. Some No. 120 mesh brass screen has recently been obtained which has a mesh aperture rated at 0.117 millimeter, but varying from 0.103 to 0.120, according to the writer's measurements. This promises to be a very satisfactory substitute for the bolting cloth. Presumably it will retain its mesh aperture, and will be more durable.

When the shot are used, they are poured with the feces into the coarsest of the brass sieves. They are subsequently poured from the sieve into a petri dish with whatever coarse fecal matter may accompany them and the fecal matter easily removed by a stream of tap water. It might be supposed that fecal matter of some sort would stick to the shot, but it does not do so. Parasitic material that might adhere can be destroyed by dry heat. Lead shot have the advantage over steel shot that they may be kept in a formol solution without rusting, while steel shot would need careful drying, or else keeping in oil.

Where small amounts of feces are used, two pieces of brass tubing, 2 inches in diameter and 1¼ inches high, beveled to fit one in the other, and the lower one furnished with three projections to hold it on glassware of not over 3 inches in diameter, are used to hold the bolting cloth of the silk sieve, the cloth being caught and held taut by the beveled surfaces. Where large amounts of feces are used, and large amounts—not to exceed 4 or 5 ounces—should be used whenever obtainable, two enameled tin pans, with a bottom diameter of 4½ inches and with the bottom cut out so as to leave a narrow flange, are used in place of the brass tubing. The cloth is held between the upper flanges of the two tins, and the cloth and flanges held together by four small clothespins of the sort provided with a wire spring to hold the jaws together. This device I also owe to Dr. Cobb. The sieve formed of the tins and the bolting cloth is the right size for use with a jar 10 inches high and 5 inches in diameter (see fig. 1, *c*). When necessary a soft brush is used to brush the feces through the fine brass sieves or the silk cloth. Cobb (1904), as previously noted, uses a brush for the same purpose. These sieves will work better if water is poured through them or if they are dipped in water before the fecal matter is poured on.

The sieves, as well as all other apparatus coming in contact with the fecal material, are washed promptly with boiling water, the sieves being also scrubbed with a stiff brush. This prevents any parasitic material from remaining to contaminate a subsequent fecal specimen, and thereby giving inaccurate findings. A microscopic examination of the silk sieve shows that it washes clean very readily, and when rinsed retains very little of the material poured on it. Experiment shows that eggs pass through this cloth very readily, less than half of 1 per cent of even such large eggs as *Toxocara* remaining when the fecal matter is first brushed through. A smear made from the residue on the bolting-cloth sieve showed 4 eggs in one case where the slide preparation from the centrifuged sediment showed 860 eggs; another smear from the residue on the bolting cloth showed 1 egg where the slide from the sediment showed 475 eggs. In the latter case, a fair estimate of the amount of material on the cloth and the amount from which the smear was made indicates that there were probably not more than 10 eggs left on the cloth, while thousands had passed through it. Those that are left are held by the jelly-like residue obtained at this point, and not because the screen mesh is defective or too small. The writer is not aware of any parasite eggs which are too large in their smaller diameter to pass through a mesh with a diameter of 0.117 to 0.134 millimeter. The number remaining after clean water has been poured through the cloth into the jar is entirely negligible.

In working with human feces, or where dangerous infection may be present, the silk cloth may be kept in a jar of formol solution when not in use. In the course of a large number of experiments nothing has yet indicated that parasitic material from one examination has remained to subsequently contaminate other fecal specimens. Parasites that might be suspected of remaining after the cloth had been washed in boiling water might be destroyed by prolonged boiling or subjection to dry heat—experiment shows that eggs so treated are distorted or characterized by the formation of air spaces or oily areas—or fresh pieces of cloth could be used each time. This last, however, would be somewhat expensive, as this cloth retails in Washington in half-yard widths at about $5 a yard. It would be cheaper to use the No. 120 mesh brass screen. This costs $1.85 a square foot, but would be permanent.

SEDIMENTING AND CENTRIFUGING.

The feces which pass through the silk sieve are sedimented with plenty of water in the jar. After decanting, the sediment is transferred to a beaker (fig. 1, *d*) and may now be washed if desired. The entire sediment, or as much as seems desirable, is then centrifuged (see fig. 1, *e*), repeated centrifuging with the addition of fresh material adding to the total centrifuge sediment, and may be washed at this point also, as advised by Pepper (1908) and Bass (1909). The writer sometimes washes the material at both points, the second supplementing and completing the first. It is usually sufficient to wash the sediment in the centrifuge. Bass has called attention to the important fact that a centrifuge should only be run the minimum time necessary to bring down the eggs. This time will vary with different centrifuges. With a centrifuge running 3,500 revolutions per minute Bass allows 4 to 10 seconds. I find this enough time with a centrifuge running 1,230 revolutions per minutes. After the material in the two centrifuge tubes is washed in water, one tube is left alone; the water is poured off the other and calcium chlorid solution, with a specific gravity of 1.250, is added to the sediment. After centrifuging, a slide preparation is sometimes made from this tube direct. In most cases the top cubic centimeter is pipetted off, shaken up with 14 cubic centimeters of water, and centrifuged. This is the more satisfactory and certain method.

PREPARATION OF SLIDES.

By means of a long pipette, a drop of sediment is drawn up from the bottom of the tube in which water alone is used, placed on a slide under a cover glass, and examined with a microscope (fig. 1, *f*). A second slide is made from the other tube. This second slide is either made directly from a drop taken from the surface of the calcium chlorid solution, or from the bottom in case the top cubic centimeter has been added to water and centrifuged. The second slide is used as a check on the first. It sometimes has fewer eggs, especially when pipetted direct from the top, but it is a cleaner preparation, is easily examined, will sometimes have more eggs, especially if made from the sediment where the top cubic centimeter of the 1.250 solution has been centrifuged with the addition of water, and occasionally throws additional light on the material under examination. The pipettes are rinsed thoroughly, and when dried are heated in a Bunsen flame for a short time to destroy any eggs that might adhere, thus preventing contamination in subsequent examinations.

CONCENTRATION OBTAINED BY THE USE OF SIEVES.

In examining the feces of 35 sheep, the entire amount of feces passing through the sieve was centrifuged in order to give a uniform comparative study and to determine the amount of concentration attained by the use of sieves, and due to them alone. To eliminate other factors, the sediment was not washed and the centrifuge was run for long periods till everything had come down. Centrifuging the entire amount of feces necessitated the repeated filling of the tubes of a two or four arm centrifuge. A comparative examination of slides made from the sediment obtained by centrifuging a single tube full of the material, with slides made from the total sediment, showed that the concentration was the same in both cases, a result which would be expected from a theoretical standpoint. While the concentration is the same, the total amount of parasitic material present is, of course, much less in the single tube.

Using moist fecal pellets, the concentration obtained was 4:1. The concentration varies with animals of other species, with food habits, and with the condition of the particular fecal specimen examined. At the same time, the concentration is always sufficient to warrant the use of the sieves. The microscopic field obtained after treatment of feces in this way is very much more satisfactory than the field obtained in the smear method, and where the same number of slides are examined the likelihood of finding evidences of existing parasitism is certainly more than four times greater in cases where the feces have been subjected to thorough sieving.

SUMMARY OF METHOD.

The writer's method is, then, merely a modification of existing methods, and might be termed a comminution-sieving-sedimentation-centrifuge method in which water alone is depended on as a medium for these operations, a slide made after centrifuging in a calcium chlorid solution with a specific gravity of 1.250 being regarded principally as a check on the method as given.

COLLECTION INDEX.

The writer has had no opportunity to work out an index showing the relation of the count obtained by the writer's method to the number of worms present. In few cases where the feces of animals are examined in this laboratory are the animals examined post-mortem within a short time. In one instance, 3 grams of feces were taken from the rectum of a sheep during a post-mortem examination for parasites, and after treatment by the writer's method, noting the time element and the amount of water used in each case—which data need not be given here—a slide was made of one-tenth of a cubic centimeter of sediment. This slide showed 3,325 nematode eggs and embryos. The intestines and fourth stomach showed a correspondingly high degree of infection with numerous species of worms, and it was impossible to determine the relation between the number of female worms of any species and the number of eggs of that species under the circumstances.

In spite of the relation found by Cobb (1904) as regards flukes, by Leichtenstern and by Grassi and Parona, according to Dock and Bass (1910), as regards hookworms, between the number of parasites present and the number of eggs in a given fecal sample, the examinations made by the writer do not indicate that anything of the sort is apt to prove of use in routine examinations for parasites in general. Dock and Bass (1910) have come to similar conclusions and cite the work of Ashford and King to the same effect. Not only is there a certain element of chance in the detection of eggs when they are present in light infections, but there are certain conditions which permit of the existence of parasitism without the presence of eggs in the feces to indicate it. Some of these conditions are as follows:

(1) Infection with male nematodes will not be indicated by eggs in the feces. Occasionally the only nematodes of a given species which are present in an animal will be males. Such infections are usually light.

(2) The above condition will not be true of the flukes and tapeworms, as they are hermaphroditic; but in the case of these worms, and the nematodes also, infections which are so recent that the infecting larvæ have not yet reached the egg-producing stage will not be indicated by eggs in the feces. Such infections may be heavy.

(3) Egg production in female or hermaphroditic animals may be interrupted in various ways. Thus tapeworms may be broken, perhaps by intestinal peristalsis, at points anywhere from just back of the head to just in front of the gravid proglottids, and the feces will show no more eggs or proglottids until new gravid proglottids are formed. Dock and Bass (1910) report that in an examination of 247 female *Necator americanus* by Bass, 7 per cent were found to

contain no ova. Bass suggested the possibility of old age as an explanation of this. Dock and Bass (1910) state that they found 9.25 per cent of 397 female *N. americanus* barren in cases where the infection was of long standing, while only one-third of 1 per cent of 120 female hookworms from the dog were without eggs. They state, however, that Dr. Stiles does not think that hookworms live long after old age induces cessation of ovulation.

(4) A thorough purging of the intestinal tract may remove an accumulation of parasite eggs, with the result that numbers of eggs will be found at this time and none a day or two later. Dock and Bass (1910) comment favorably on Ashford and King's statement that hookworms cause an increase of mucus at the site of their feeding ground, and as this mucus often comes off en masse and contains most of the eggs the actual feces would contain relatively few. They also note that Ashford and King say that eggs are more difficult to find in diarrheal stools, and that they saw cases of heavy infection that at times had no ova in the feces. Dock and Bass (1910) call attention to the fact that the amount of feces varies with different persons and from time to time in the same person. They also state that it is a common experience to find no eggs in the feces within a few days after a course of thymol and subsequently to find many eggs. They say that Dieminger found that the number of eggs was very much diminished when patients were drinking hard.

It follows from the above that little weight can be laid on a collection index. In the writer's experience, consecutive and careful examinations of the feces of a given animal show days when eggs of various kinds are abundant and days when they are scarce or missing, and the collection index for the various days would present a striking disagreement. This would be particularly true of cestodes.

Negative examinations must be considered doubtful and must be checked at intervals if infection is suspected.

TESTS OF UNPUBLISHED METHODS.

Numerous tests of unpublished modifications of technique were made in connection with the development of the writer's method. Among these was an attempt to increase the specific gravity of parasites and their eggs by adding mercuric chlorid before centrifuging. Comparative tests did not indicate that this method was of value, and any adaptation of technique which would make it so would probably not repay in results what it required in time. Tentative attempts to use a differential stain were unsatisfactory. In one of these, sulphuric acid and iodin were added to slide preparations to distinguish cellulose substances which might otherwise have to be examined as possibly parasitic. By this method cellulose was colored violet or black, and nematode eggs brown, or at times black, with a light areole where the shell showed at the periphery. As such a method lessened the refractive index which makes most nematode eggs so conspicuous, the use of these reagents was discontinued as giving no improvement in the resulting preparations. Stubbendorff (1893a) has noted this test and a number of others in a note on the differential diagnosis of parasite eggs and plant spores.

COMPARATIVE TESTS OF THE WRITER'S METHOD AND OTHER METHODS.

For about a year comparative tests of the writer's method and other methods were made in the examination of feces of various kinds. So far as possible equal amounts of feces were examined and the slides made from equal amounts of material. The parasite eggs and embryos were carefully counted with the aid of a mechanical stage, and the results compared.

SMEAR METHOD.

Comparative tests of the smear method and the writer's method, some results of which are given in connection with other tests, indicate that the smear method is much less certain and effective. A diagnosis made by the writer's method would be much more complete and adequate than one based on the usual ten smear preparations.

SEDIMENTATION METHOD.

The simple sedimentation method is so obviously inferior to methods involving sieving and centrifuging, and improved modifications of the sedimentation method were found so inferior to the writer's method in actual test, that the simple method was not even given a comparative test.

BURETTE METHOD.

A test of the sedimentation method in which the sediment was taken from the bottom by means of a stopcock did not give as good results as the centrifuge method. In this test a burette of 25 cubic centimeters capacity was used. Ten cubic centimeters of water were first put in it, with the idea of washing the feces at the bottom of the burette instead of at the top, as is commonly done where water is poured over a sediment and the sediment shaken up in the water, allowed to settle, and the fluid then decanted. It appeared that in this way the sediment could be taken out promptly and already washed. In testing, the sediment obtained after screening and decanting was shaken vigorously and divided into two equal parts, one being centrifuged and washed, the other being poured onto the 10 cubic centimeters of water in the burette. As the sediment settled it could be traced by the discoloration of the water, and when the discoloration reached the bottom one preparation was made by opening the stopcock and taking a drop of water on the slide. Other slides were made at intervals up to an hour. In one specimen of dog feces where there was an infection with *Dipylidium*, *Ascaris*, *Trichuris*, and *Ancylostoma*, the best of six slides made in this way showed a total of 27 eggs, while a slide made after centrifuging with calcium chlorid of 1.250 specific gravity showed 77 eggs and one centrifuged in water alone showed 181 eggs. In the last case there were more eggs of any one of the four species of parasites present than there were of all together in the best of the slides made by the burette method.

In another test, feces from a dog infected with *Toxocara* and *Tænia* were tested by the burette method without the clean water at the bottom, the sediment obtained after screening being poured into the empty burette. The best of two slides made by this method showed 58 eggs, a slide made by the smear method showed 46 eggs, a slide made by centrifuging in the solution of 1.250 specific gravity showed 117 eggs, and a slide made after centrifuging in water alone showed 215 eggs in one-fourth of the cover-glass area. The slide started to dry at this point and the count was discontinued. The estimated total for the slide was of course four times 215, or 860 eggs. A smear preparation made from the residue on the bolting-cloth sieve showed four *Toxocara* and no *Tænia* eggs.

GASTEIGER'S FILTER METHOD.

A test of the filter paper and funnel as a substitute for the centrifuge indicated at once that nothing was gained by scattering a sediment over a filter paper instead of concentrating it in the bottom of a tube. The method was also slow and offered the usual chance of a break in the filter paper, necessitating a second filtration. I can not imagine any use of the filter in fecal examinations for parasites that would not be better subserved by the use of the sieve followed by centrifuging.

BASS'S CALCIUM CHLORID CENTRIFUGE METHOD.

Inasmuch as Bass's salt solution method is held by its author to be inferior to the calcium chlorid method, it need only be noted here that in the few tests of the first-named method made by the writer its inferiority to the calcium chlorid method and to the writer's method was quite apparent.

In comparative tests of Bass's calcium chlorid method, extending over a number of months and involving the examination of the feces of man, the dog, sheep, eland, hartbeest, and chicken, it was found that this method secures a high degree of concentration of material, but in eliminating nonparasitic material it also eliminates some parasites. Part of these seem to be left in the rejected sediment at the bottom of the tube containing the solution with a specific gravity of 1.250, as repeated examinations of this rejected sediment showed numbers of parasite eggs and embryos in nearly all cases, indicating that endosmosis brings the specific gravity of the parasites up to that of the surrounding fluid. Some parasites seem to be lost also in the repeated handling due to the use of the solution with a specific gravity of 1.050, as more parasites were recovered where the use of this solution was omitted and the 1.250 solution alone used than where both were used, as Bass directs. Where slide preparations were made from equal amounts of sediment secured by Bass's method and the writer's method, the amount of parasitic material to a slide was usually greater when prepared by the writer's method. There is, furthermore, a distortion of parasite eggs and embryos due to osmosis. This distortion and destruction of parasites constitutes at once a limitation and a defect of the method. It renders it unsuitable for the collection of live material and makes it more difficult to recognize and identify some parasites, such as nematode embryos.

In an examination of some human feces from a case of hookworm infection, which material was obtained through the courtesy of Dr. Stiles and his assistant, Mr. Crane, a smear preparation of the feces showed 2 eggs, a preparation made by Bass's method showed 84 eggs, and a preparation made by the writer's method showed 348 eggs. An explanation of the failure of Bass's method to give as good results was found in an examination of the sediment in the tube containing the strong calcium chlorid solution. A preparation made from this sediment showed 122 eggs.

In an examination of the feces of a number of dogs, the feces were treated according to the method given by the writer, according to that of Bass, and according to Bass's method without the use of his solution of 1.050 specific gravity. The method involving the use of the 1.250 solution, but not the 1.050 solution, is given in the table below as Bass's modified method. The slides were made by drawing up definite equal amounts of sediment in a graduated pipette from the bottom of the centrifuge tube. The results of the tests are given in the following table. Generic names refer to eggs found.

Comparative tests of three methods in examination of dog feces.

Feces.	Method.		
	Writer's.	Bass's.	Bass's modified.
	Eggs.	*Eggs.*	*Eggs.*
Dog No. 1	1 Nematode embryo 8 *Ancylostoma*	Negative	Negative.
Dog No. 2	28 *Ancylostoma* 28 *Trichuris*	5 *Ancylostoma* 18 *Trichuris*	23 *Ancylostoma.* 3 *Trichuris.*
Dog No. 3	Negative	4 *Toxocara*	Negative.
Dog No. 4	13 *Ancylostoma*	10 *Ancylostoma* 1 *Toxocara*	16 *Ancylostoma.* 1 *Toxocara.*
Dog No. 5	20 *Ancylostoma*	3 *Tænia* 10 *Ancylostoma*	36 *Ancylostoma.*
Dog No. 6	4 *Toxocara*	1 *Toxocara*	2 *Toxocara.*
Dog No. 7	258 *Tænia*	209 *Tænia*	299 *Tænia.*

An examination of the above table shows that the results obtained by the use of the writer's method and of Bass's method without the step involving the use of the solution of 1.050 specific gravity, are on the whole superior to those obtained by the use of Bass's method. Dock and Bass (1910) state of Bass's method:

> Gage and Bass conclude, after this extensive experience [the examination of the feces of 315 students by the smear method, by sedimentation and centrifuging, and by Bass's method], that for all practical purposes washing with water alone is all that is necessary and that the washing with calcium-chlorid solution is unnecessary except for special purposes.

The fact that the use of the strong calcium chlorid solution gives at times better results than the use of water alone is the writer's warrant for making use of it in one centrifuge tube to check the findings from the other tube.

GARRISON'S CALCIUM CHLORID SEDIMENTATION METHOD.

Garrison's method of using calcium chlorid and sedimenting instead of centrifuging has come to the writer's attention too recently to permit of adequate tests. A test made by sedimenting in a centrifuge tube instead of centrifuging did not give as good results as centrifuging. Sedimenting has the disadvantage of being slower than centrifuging.

TELEMANN'S ETHER HYDROCHLORIC ACID METHOD.

A number of tests were made to determine the applicability of Telemann's (1908) chemical methods to the examination of feces. Telemann himself states that he has found his method satisfactory in a large series of human and animal feces. He does not specify what animals are included in the series, but his statement may be believed in any case. At the same time, the tests made in this laboratory indicate that his success was due more to the use of the hair sieve than to the use of chemicals. The latter probably did more in mechanically breaking up the feces by a slight amount of chemical action than in actually concentrating parasitic material by eliminating nonparasitic matter soluble in ether and hydrochloric acid. As previously stated, the tests of the writer's method on sheep feces gave a constant theoretical concentration of 4:1. The addition of ether and hydrochloric acid to a given 0.5 cubic centimeter of sediment so obtained gave a reduction to 0.4 cubic centimeter, or an additional theoretical concentration of only 5:4. When the ether and hydrochloric acid were added to the fresh feces a somewhat smaller sediment was obtained. This does not indicate a greater concentration, as it appears to, but a lesser breaking up of feces by these reagents than by water, so that more agglomerations of material that should be broken up to allow the finer matter to pass the sieve are left unbroken, and the small as well as the large particles are held by the sieve. This is evident from the result obtained by adding these reagents last to determine the action due to them alone.

Another result sometimes obtained by using Telemann's method on sheep feces was that a plug of plant material formed at the top of the centrifuge tube and held in its mesh almost all the fecal material. The feces of herbivora are composed largely of plant matter and hence largely of cellulose. This is removable to a great extent by sieving but is not at all soluble in Telemann's reagents. This is also true of the feces of such birds as chickens and pigeons, as tests with such feces indicate.

On the other hand, the feces of man and of the carnivora have less plant matter of the sort and more matter that is soluble in ether and hydrochloric acid. Nevertheless, comparative tests indicate that the writer's method is practically as good as Telemann's for these feces also. In tests with human feces where the writer's method gave a theoretical concentration of 4:1, Telemann's method gave the same result. In one case the sediment obtained by the writer's method was treated by Telemann's method, and after shaking up and stirring was again centrifuged. The resultant sediment showed no reduction in volume as a result of the action of the chemicals. In another case where the writer's method gave a sediment of 0.85 cubic centimeter, an application of Telemann's method to this sediment reduced it to 0.65 cubic centimeter. Even more surprising results were obtained with dog feces, used as representative of feces of the carnivora. In test cases sieving in water gave a concentration of 5:1. In one case the application of Telemann's method to the sediment so obtained reduced it to half of its volume, thereby doubling the concentration. Although the concentration was doubled, the fact that the writer's method had reduced the original 1 cubic centimeter to 0.2 cubic centimeter, thereby eliminating 0.8 cubic centimeter, while the chemicals had only reduced it by 0.1 cubic centimeter more, indicates that the additional solution of material accomplished by the use of these chemicals was but slightly more than the solution accomplished by water alone. In two other cases where dog feces had been allowed to stand a long time in water and had been shaken up for four or five minutes before being sieved and centrifuged, the resultant sediment of 1.4 cubic centimeters was actually increased after the application of Telemann's reagents followed by centrifuging to a flocculent sediment of 1.9 cubic centimeters.

The results of comparative tests of Telemann's and the writer's method were quite unexpected, as the writer had used Telemann's method for a year and only abandoned it when its inapplicability in the case of sheep feces became too evident to overlook. As has been stated previously in this paper, the success of Telemann's method appears to be due more to the efficiency of a slight solvent action in mechanically breaking up feces and putting them in condition to sieve, than to an extensive solvent action. Much that might be dissolved in the chemicals is soluble in water, much that might be dissolved is perhaps taken out by the screen where water alone is used, and the fact that the slight solvent action breaks up the feces and makes them easy to sieve seems to be the feature to which the results obtained by this method are to be attributed.

The feces of herbivora have little material that is soluble in Telemann's reagents, and the breaking up secured by them is inferior to that secured by the use of the writer's method. Human feces break up rapidly in Telemann's reagents, but the method advocated by the writer takes about the same time and gives as good results. Of Telemann's method, Dock and Bass (1910) say:

> We have found the method admirable with some stools, but in others not enough solution occurs to permit a concentrated layer of eggs to be thrown down.

Telemann's method has one disadvantage which neither Telemann (1908), Pfister (1909), nor Quadflieg (1909) mentions in connection with it—it injures the microscope. The fumes of pure hydrochloric acid attack the lens mountings and also the stage and the lenses themselves. The use of vaseline to seal the edges of the cover glass affords an uncertain protection, and the use of high powers under such circumstances is decidedly unsafe. In the writer's opinion the injury to the microscope would of itself be sufficient reason for discarding this method.

CULTURE METHODS.

Quadflieg (1909) has recommended using culture methods as an aid in detecting infection. Tests of this method did not indicate that it added to the certainty of detecting infection, though live embryo nematodes could be detected by the use of a dissecting microscope with low-power lenses. As the determination of nematode genera and species is in general more difficult from the embryos than from the eggs, the usefulness of the culture method is limited.

Emerson (1910) uses the following method in searching for *Strongyloides intestinalis:*

> The stool should be placed in a dish, a small depression made on its surface, this filled with water, and the stool left in a thermostat overnight. If embryos are present, they are easily found * * * actively swimming in this water.

Culture methods have been used by Ransom (1906i) in studying *Hæmonchus contortus* in sheep feces. Dr. Garrison tells me that he has seen the culture method used successfully in Army work in examining a company for hookworm infection.

It is evident that such methods are suitable only for special purposes and not for general work.

OBJECTIONS TO CERTAIN METHODS.

The reason for rejecting some of the methods outlined in the hypothetical ideal treatment previously mentioned is, as was stated, that in actual practice objections of two general kinds may be urged against them. These objections are: (1) The injury to parasites or eggs; (2) the unsuitability of the methods for certain kinds of feces or the fact that the concentration resulting from the application of the method may be too insignificant to warrant the time and effort expended. It will be fairly evident from the discussion of methods already given just how those methods fail in these two respects, but a brief summary will indicate some general truths.

INJURY TO PARASITES.

In regard to the first point, it is evident that if parasite eggs or embryos are to be used in feeding experiments to produce infection, or are desired alive for study, or if the movement of embryos is depended upon to indicate viability or to aid the eye in detecting or locating the parasites, certain methods, such as boiling (Cobb, 1904) or the use of chemicals which will kill by poisoning or by rapid osmosis (Telemann, 1908; Bass, 1909), can not be used. Bass's (1909) method has the further objection that it not only kills, but distorts. Letulle (1905) urges that certain eggs, such as those of *Bilharzia* and *Uncinaria*, are mechanically injured by the use of the centrifuge. I have not found this true of the eggs of the human, sheep, or dog hookworm, or any other parasites encountered, but there might be cases where the objection would hold good. The writer's use of shot would work occasional injury to parasite material and it should be avoided when possible. In almost all cases, the feces can be broken up in a little longer time by shaking in water alone, as experiments show.

LIMITED APPLICATION OR INCOMMENSURATE RESULTS.

In regard to the second point, experience shows that in using specific gravity as an aid in concentration it is safe to assume that the specific gravity of a parasite is greater than water, though all eggs will not go to the bottom, as some are held up by lighter material, bubbles, etc., but it is not safe to rely on a parasite floating on a solution of specific gravity greater than itself for any length of time, owing to the action of endosmosis. An additional objection to this method advocated by Bass (1909) is that experience shows that all the steps are not warranted by the results. In most cases the simpler methods used by the writer give as good or better results. The use of the 1.050 solution appears to be a defect of the method. The concentration attained by it seems trifling in any case and at times it apparently results in actual loss of parasite material. Finally, under the same class of objections, experience shows that the concentration obtained by the use of chemicals instead of water (Telemann, 1908) is no greater than that obtained by the use of water alone, and hence there appears to be no reason for using the more expensive chemicals, especially in view of the injury to the microscope which one of them occasions. It might also be urged that the odor of these chemicals is such that one would prefer to avoid them, though it is also true that they deodorize the feces after a fashion, and that while they kill the parasites they also disinfect the feces in cases where the killing is immaterial.

DISINFECTION OF FECES.

Disinfection and deodorization of feces can be more easily accomplished, if desired, by the use of formol solution instead of water, as suggested by Letulle (1905), Jones (1907), and Garrison (1910). In a discussion of this point before the Helminthological Society of Washington, December 1, 1910, Dr. Stiles advocated the use of coal-tar disinfectants on the ground that protracted work with formol material would cause headaches. The writer has never experienced any inconvenience from the use of formol. Dr. Stiles also pointed out the great desirability of using some disinfectant, for the reason that the greater number of fecal examinations now being made in this country are for evidence of hookworm infection, and the localities with the greatest amount of hookworm infection have a high typhoid index. In using a disinfectant, the writer's method of comminution has the advantage of bringing the disinfectant into intimate contact with all parts of the fecal mass. The thorough breaking up of the feces and the use of large amounts of water reduces the odor to a point where it is almost imperceptible in most cases.

SUMMARY.

After testing the various methods as above indicated, the writer finds that the best results in routine examinations of feces of all kinds are obtained from the simple method already given. Briefly, the method consists in breaking up the feces very thoroughly by shaking in water, adding a quantity of small shot if necessary or desirable; sieving through a set of brass sieves and then through a silk bolting-cloth sieve or a sieve made with a jeweler's fine-meshed brass screen, examining the material left on the sieve for parasites; sedimenting (and washing); centrifuging (and washing)—one tube being filled with calcium chlorid solution of 1.250 specific gravity, centrifuged, and if desired the top cubic centimeter removed with a pipette, shaken up in a tube with 14 cubic centimeters of water and centrifuged—and then making a microscopic examination of a drop of sediment from the bottom of the tube centrifuged with water, and one from the top when the calcium chlorid solution alone was used or from the bottom in case water was added to the top cubic centimeter. The material is washed at either or both of the points indicated.

ADAPTABILITY OF METHOD.

The writer does not claim that the method advocated here is the best possible method. It is, however, the method which his experience shows to be the best for routine examination of various kinds of feces after comparative tests with other methods. It serves very well for the feces of man, and of the carnivora, herbivora, and birds, so far as fecal examinations for representatives of the last three groups indicate. It is not only of service in examining feces for worm parasites, but also for coccidia. It has not been tested for other protozoa. Presumably the writer's comminution method would damage flagellates, ciliates, or amebæ. It is often useful in detecting parasitic infection of stomach and intestinal contents. It has the advantage of speed and certainty over the smear method or sedimentation methods. It takes longer to make the microscopic preparation than in the smear method, but the resulting concentration justifies it. Nor is it a long or difficult process. The time required for each step is slight—a minute to shake up the feces, another to sieve them, and another to centrifuge them, leaving out the sedimentation after sieving, which needs no attention, and the time spent in examining material on the screens, which examination is incidental to the technique, not part of it. As everyone who has used laboratory methods knows, the total time necessary to perform three one-minute operations is not just three minutes or, as a rule, even six minutes, as preparation, intermediate steps, cleaning up, etc., add considerably to the time necessary. At the same time, the method outlined here is reasonably rapid and takes less time than is required to examine the additional slides which the use of the smear method would demand. I find that the careful examination of a slide not uncommonly takes 9 or 10 minutes. Garrison, Ransom, and Stevenson (1903a) state that the examination of 100 preparations is an average day's work for one person. This is a little over four minutes for each preparation. Stiles (1909) states that a thorough examination of 10 slides will take 40 to 60 minutes, or 4 to 6 minutes for each preparation. Dock and Bass (1910) claim to look over an ordinary slide thoroughly in 2 to 4 minutes. If one slide prepared by the writer's method be considered as equivalent to 4 smear preparations, and experience shows that it is much better than this, then it will save time to spend 8 minutes in obtaining a sediment and 8 more in examining 2 preparations made from this sediment, rather than spend 32 minutes examining 8 smear preparations. Where positive information as to parasitic infection is desired, the best methods and the time necessary for these methods are abundantly warranted. Dock and Bass (1910) state that Gage and Bass, in the examination of the feces of 315 students, found only 47 per cent of the cases of intestinal parasitism by the smear method, the remaining 53 per cent being found by the use of centrifuge methods. They note cases where the examination of 25 smear preparations failed to show infection, although the existence of infection in these cases was demonstrated by preparations made after sedimenting and centrifuging.

Only in those cases where the necessary apparatus for better methods is not available, or where evidence of heavy infection is sought for with a view to immediate medical treatment, would the writer consider the routine use of the crude smear method warranted. In exceptional cases its use might be warranted in examining one or two slides for specific infection where there is likelihood of the infection being heavy enough to be promptly discovered by this method.

ECONOMY OF METHOD.

If the time of a physician, veterinarian, or scientist is of any value, then the smear method is not even more economical than the writer's method. The centrifuge is a thing which the workers just mentioned should have for purposes other than fecal examinations, and the only additional pieces of apparatus required—the screens—are inexpensive and durable.

CONCLUSION.

The method of examining feces for evidences of parasitism which consists in putting the feces through a process of thorough shaking, sieving, sedimenting, and centrifuging appears, from a theoretical standpoint and in actual experience, to give the best results in the shortest time and with a minimum financial expenditure when the value of time saved is considered. It is therefore advocated as a practical routine method of examining feces of all sorts.

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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 136.

A. D. MELVIN, CHIEF OF BUREAU.

THE DIAGNOSIS OF GLANDERS BY COMPLEMENT FIXATION.

BY

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WASHINGTON:
GOVERNMENT PRINTING OFFICE,
1911.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., March 20, 1911.

Sir: I have the honor to transmit herewith a paper entitled "The Diagnosis of Glanders by Complement Fixation," by Drs. John R. Mohler and Adoph Eichhorn, of the Pathological Division of this bureau.

Since the discovery of the glanders bacillus in 1883 many efforts have been made to find a reliable method of making an early diagnosis of the disease. The mallein test and later the agglutination test have been and are at present in general use, but neither of these is sufficiently reliable to be entirely satisfactory. Schütz and Schubert, German investigators, recently called attention to the value of complement fixation as affording a more reliable method of diagnosing glanders, and within the past year this method has been carefully studied and tested in the Pathological Division of this bureau.

It will be seen from the details given in the accompanying paper that the authors have found the complement fixation test to be highly reliable as a diagnostic agent for glanders, and they present a thorough exposition of the method resulting from their searching experiments, including practical tests in a recent outbreak of glanders at Washington, D. C.

In view of the great economic and scientific importance of the subject, and as no work upon this new method has so far been published in the United States, I recommend the immediate publication of the paper in the bulletin series of this bureau, in order that the value of the method and the technique necessary for its application may be made more fully available in this country.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

	Page.
Introduction	5
Hemolysis	7
Method of obtaining hemolytic amboceptor (rabbit serum)	9
Titration of hemolytic rabbit serum	12
Method of obtaining complement (guinea-pig serum)	14
Titration of complement	14
Specific complement fixation (deviation)	16
Method of obtaining serum of animals to be tested	18
Preparation of the antigen (glanders bacilli extract)	19
Titration of the extract	20
The complement fixation test	22
Application of the test	23
Controls	24
Interpreting results of tests	26
Controlling glanders in an infected stable	28
Results of practical tests with complement fixation	28

ILLUSTRATIONS.

	Page.
PLATE I. Diagrammatic representation of complement fixation	8
II. Titration of hemolytic amboceptor (rabbit serum)	12
III. Titration of complement (guinea-pig serum)	16
IV. Titration of antigen (glanders bacilli extract)	20
V. Final test showing positive reaction to glanders	26

THE DIAGNOSIS OF GLANDERS BY COMPLEMENT FIXATION.

INTRODUCTION.

The early diagnosis of glanders constitutes one of the most important and difficult tasks which confronts the veterinarian engaged in sanitary work. This of course does not apply to the clinical cases of glanders, as in such cases the diagnosis is usually made without much difficulty from the characteristic symptoms and lesions present. In those instances, however, where there are no positive indications of the disease, it is impossible to establish a diagnosis by physical examination, and only through the aid of some special diagnostic method or test can there be any hope of determining the presence or absence of the disease. Horses affected with occult or latent glanders, and in which the disease is not suspected, are undoubtedly great factors in the propagation of the infection. Indeed, there are many glandered horses which do not show positive symptoms until the later stages of the disease.

Since the discovery of the glanders bacillus in 1883 by Loeffler and Schütz the diagnosis of glanders has been the subject of numerous investigations, and as a result great progress has been made in its determination. After the isolation of the infective agent of the disease the diagnosis was confined to the demonstration and cultivation of the organism, or to the reproduction of the disease by inoculations of exudates or parts of diseased organs from affected horses into susceptible animals.

The first important step toward determining obscure and latent cases of glanders was made by the discovery of mallein. With the aid of this biological product of the *Bacillus mallei* a large proportion of latent and occult cases of glanders can be diagnosed, particularly when such tests are made by efficient and experienced veterinarians. There are, however, a considerable number of glanderous animals in which the mallein fails to give a typical reaction, and, on the contrary, a reaction may follow the injection of mallein in the absence of glanders. Thus mallein is not an entirely reliable diagnostic agent for determining glanders, nor has it ever been considered as efficacious in the detection of this disease as tuberculin for the diagnosis of tuberculosis.

With the application of the agglutination test for glanders it appeared that a more satisfactory method had been found for the diagnosis of all types of infection with this disease. It was first suggested by McFadyean in 1896, after this investigator had observed the value of Widal's typhoid-fever agglutination test, but was not generally adopted until the method was perfected by Schütz and Meissner, whose interesting results were published in 1905. This test has since been extensively employed in practically every country where glanders exists, and therefore ample opportunity has been furnished for drawing conclusions relative to its diagnostic value.

While there is no doubt that the agglutination test is of great value in all cases of recent infection, the blood in such cases possessing a very high agglutination power (1 to 1,000 and higher), nevertheless extensive experience has proved that horses affected with chronic glanders give occasionally a very low agglutination value, which in some cases is even lower than that of normal blood serum (1 to 400 or even lower). From this condition it appears evident that in certain cases of chronic glanders the disease can be determined only by repeated tests, and a diagnosis in such cases is only possible from the fluctuation of the agglutination value—either an increase or a decrease—as it is a well-known fact that this value remains stationary in normal horses.

Besides this difficulty, there should also be taken into consideration the fact that the blood of normal horses sometimes shows a high agglutination value (1 to 800 and higher), and that changes in the agglutination power have been observed even in animals free of glanders. Furthermore, repeated agglutination tests require considerable time, as at least two weeks should elapse between two tests. Therefore the agglutination test alone does not constitute an entirely satisfactory diagnostic method for glanders. However, as its great value has been proved beyond doubt in the early cases of infection, it may well be utilized as an adjunct to any other test which may be applied in connection with the diagnosis of suspected cases of the disease.

Hutyra compared the agglutination test with the mallein test from the tables included in the works of Schütz and Miessner and of Nevermann, and came to the conclusion that the application of the agglutination test alone has not decreased the number of faulty diagnoses. He believes that the principal difference in the results lies in the fact that a large number of horses which were classified as only suspicious by the mallein test are considered as actually infected by the agglutination test.

In further efforts to find a method by which an early diagnosis of glanders could be made, various investigators directed their attention to the precipitation reaction. This is based upon the fact that when blood serum comes in contact with a concentrated extract of glanders bacilli the precipitins or receptors, which are formed in the blood of infected animals from the time the infection first occurs, are bound to the bodies in the bacillary extract, producing a precipitation which is manifested by cloudiness at the point of contact of the two fluids. This method of diagnosing glanders has recently been recommended by Pfeiler[1] in Germany and by Konew[2] in Russia, but it has not been applied extensively in practice. This is probably due to the fact that the reading of the reaction is in some cases difficult, due to the indistinct ring which occasionally is formed at the line of contact between the precipitant and the serum.

In 1909 Schütz and Schubert[3] published the results of their important work on the application of the method of complement fixation for the diagnosis of glanders. And since their experiments were followed by splendid results, exceeding by far the results obtained from either the mallein or the agglutination test, they recommended that this method of diagnosis in combination with the agglutination test be taken as the official test in Germany. This method, overcoming as it does the disadvantages of the mallein and agglutination tests, constitutes without doubt the most reliable method for the diagnosis of glanders which we have at our command at the present time. The complement-fixation test is, in fact, the most definite method known for determining specific infections and is as nearly perfect as a biological test can be. It has only recently been introduced in veterinary science and the publications concerning it are at present limited exclusively to foreign periodicals. The principle of this test is presented in the phenomenon of hemolysis, which was first discovered and studied by Bordet and Gengou, and extended by Ehrlich, Morgenroth, and Sachs.

HEMOLYSIS.

It is a well-known fact that if red blood corpuscles of one animal are introduced into another of a different species the blood of the latter acquires the power to dissolve the blood corpuscles of the former when mixed with them in a reagent glass. This reaction is termed hemolysis, which means the dissolution of blood corpuscles, thereby setting the hemoglobin free in the medium in which the corpuscles are suspended.

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[1] Pfeiler, Willy. Die Ermittelung der Rotzkrankheit durch die Präzipitationsmethode. Archiv für Wissenschaftliche und Praktische Tierheilkunde. Band 35, Heft 4/5, pp. 323–337. June 24, 1909.

[2] Konew, D. Präzipitationsreaktion als diagnostische Methode beim Rotz. Vorläufige Mitteilung. Centralblatt für Bakteriologie. Abt. 1, Orig., Band 55, Heft 3, pp. 251–253. July 9, 1910.

[3] Schütz and Schubert. Die Ermittelung der Rotzkrankheit mit Hilfe der Komplementablenkungsmethode. Archiv für Wissenschaftliche und Praktische Tierheilkunde. Band 35, Heft 1/2, pp. 44–83. 1909.

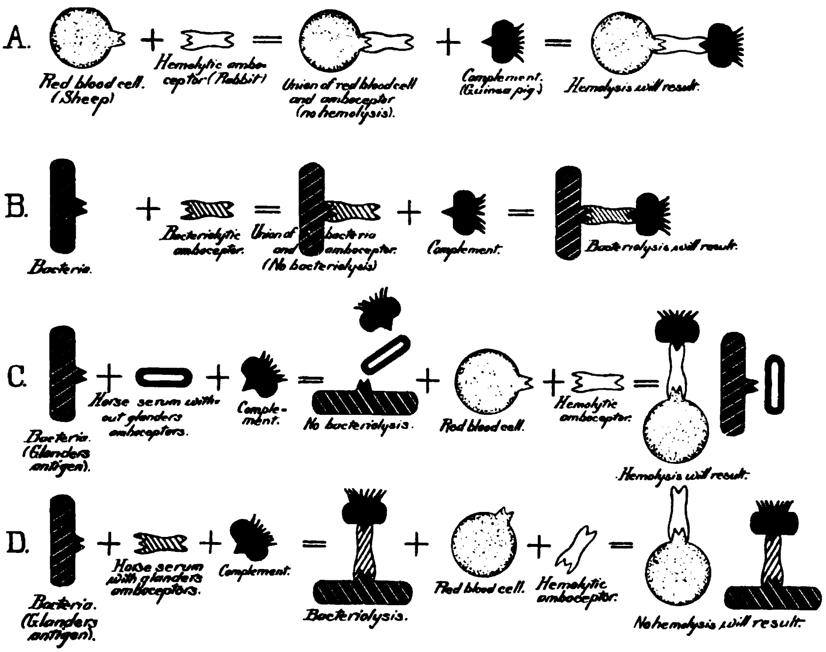
To illustrate this phenomenon, if a rabbit is injected intraperitoneally, intravenously, or subcutaneously with washed red blood corpuscles of a sheep, the blood of the rabbit will develop antibodies which possess a dissolving action for the sheep blood corpuscles; that is, the rabbit blood will contain specific hemolysins.

The acquired hemolytic property of the blood depends on two substances. One of these is present in the blood of every animal, and is known as the complement. It is thermo-labile, which means that it is rendered inactive after the blood or serum has been heated to 56° C. for half an hour. The other body, which is formed as a result of the injection of blood corpuscles, is thermo-stabile; that is, it resists heating even higher than 56° C., and is known as immune body, fixative, sensitizer, or hemolytic amboceptor. The name amboceptor is derived from the fact that it has an affinity on the one hand for the blood corpuscles of the species of animal with which the animal has been injected, and on the other for the complement, this union taking place only after the first-mentioned affinity has been satisfied.

These two substances, together with the corpuscles to be dissolved, comprise the hemolytic system, and their combination leads to hemolysis. (See Pl. I, A.) This means that an opaque suspension of blood corpuscles is rendered semitransparent or "laked." The hemolysis, strictly speaking, does not represent a complete solution, but only an action of the hemolysin on the stroma of the erythrocytes, which permits the escape of the hemoglobin of the red blood corpuscles.

The injection of blood corpuscles of one animal into another of a different species gives rise to the development of antibodies which confer upon the blood serum the hemolytic action. This phenomenon is somewhat similar to the production of receptors in the formation of antitoxins which are thrown off, but these receptors alone are not able to dissolve the red blood corpuscles, requiring also the presence of a ferment. This ferment, however, is a constant constituent of the blood and is known as the complement.

That both of these substances are constantly present in the hemolytic serum can be demonstrated in the following manner: If the hemolytic serum is heated to 56° C. for half an hour, thereby destroying the complement, this serum will no longer possess a hemolytic action; that is, it will no longer dissolve red blood corpuscles. This heating of the serum is known as inactivation. On the other hand, if to such inactivated serum there be added fresh untreated


A.
Red blood cell. (Sheep)
Hemolytic amboceptor (Rabbit)
Union of red blood cell and amboceptor (no hemolysis).
Complement. (Guinea pig)
Hemolysis will result.
B.
Bacteria.
Bacteriolytic amboceptor.
Union of bacteria and amboceptor. (No bacteriolysis)
Complement.
Bacteriolysis will result.
C.
Bacteria. (Glanders antigen).
Horse serum without glanders amboceptors.
Complement.
No bacteriolysis.
Red blood cell.
Hemolytic amboceptor.
Hemolysis will result.
D.
Bacteria. (Glanders antigen).
Horse serum with glanders amboceptors.
Complement.
Bacteriolysis.
Red blood cell.
Hemolytic amboceptor.
No hemolysis will result.

DIAGRAMMATIC REPRESENTATION OF COMPLEMENT FIXATION.

A, Hemolytic system.
B, Bacteriolytic system.
C, Negative reaction with normal horse serum.
D, Positive reaction with glandered horse serum.

serum, which in itself has no hemolytic properties, hemolysis will result. Thus by the addition of this fresh serum a reactivation is accomplished. This is explained by the fact that through the heating of the serum one of the substances necessary for the hemolysis has been destroyed, which is the complement. After the complement has been destroyed by heating it can be replaced by the addition of any normal serum, because it is known that the complement is present in all blood. However, the guinea-pig serum appears to be the most satisfactory in the application of hemolysis,.inasmuch as it is very rich in complement and only a very small quantity is required to be added to the inactivated hemolytic serum in order to produce hemolysis.

Accordingly, the substances necessary for hemolysis are (1) the hemolytic amboceptor, which is the serum of a rabbit that has been injected with washed sheep blood corpuscles, (2) the complement in the form of normal guinea-pig serum, and (3) washed red blood corpuscles of a sheep. In the preparation of these different substances it is necessary to fix standards of practical constancy by proceeding along definite lines in the following manner:

METHOD OF OBTAINING THE HEMOLYTIC AMBOCEPTOR (RABBIT SERUM).

Strong, vigorous rabbits are selected, and they are injected intraperitoneally with a suspension of washed red blood corpuscles from a sheep. Three injections are made at intervals of seven days with 7 c. c., 10 c. c., and 12 c. c. of these blood corpuscles suspended in like quantities of physiological salt solution.[1]

The sheep blood is obtained by bleeding a vigorous sheep from the jugular. The side of the neck is clipped and shaved, and the part over the jugular disinfected with 75 per cent alcohol. Then a sterilized small-calibered trocar is inserted into the jugular, and the blood is collected in a sterile bottle containing a few glass beads. After the desired quantity of blood is obtained it is shaken for 10 minutes in order to defibrinate it. After defibrination, it is filtered through a double layer of sterile gauze into the glass tube in which the washing is to take place. The glass tube containing the blood is then filled with salt solution and placed in a centrifuge which has a speed of 2,500 to 3,000 revolutions per minute. After the red blood corpuscles are thrown down the clear fluid above the corpuscles is pipetted off. Then the blood corpuscles are again thoroughly mixed with salt solution in the proportion of 1 to 9, and the centrifugalization is repeated. This washing should be carried out three or even four times in order to eliminate all the serum adhering to the red blood corpuscles. Such washed blood corpuscles can then be used for the injection of rabbits in the preparation of hemolytic amboceptors, as well as for the test proper.

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[1] Unless otherwise stated, the term " salt solution " in this bulletin refers to an 0.85 per cent solution of sodium chlorid.

The washing of the sheep blood corpuscles must be thoroughly carried out, inasmuch as the presence of even traces of serum adhering to the corpuscles may cause difficulty in obtaining satisfactory results. If rabbits were injected with red blood corpuscles containing a small quantity of serum, the rabbits would develop, not only antibodies, or immune bodies, but also coagulins and anticomplements, and the presence of these substances would give rise to difficulties in demonstrating the presence or absence of a complete hemolysis. Furthermore, if blood corpuscles containing even traces of serum were used in the tests it might produce a fixation of the complement and thereby give rise to errors. Such errors would occur particularly if the hemolytic action of the rabbit serum was not very high.

The washed blood corpuscles should be used for the injection of the rabbit on the day the blood is drawn. For testing purposes, however, it will keep for two or three days in the ice chest.

The rabbits to be injected are shaved on the posterior part of the abdomen, and the skin is disinfected with 75 per cent alcohol. They are then held with the head down by an assistant in order to prevent the puncturing of the intestines. The blood corpuscles to be injected are mixed with an equal quantity of salt solution and heated in a water bath to body temperature. Intravenous injections have been recommended by some investigators, but it was found in our work that intraperitoneal injections gave very satisfactory results, and furthermore, there is very little danger of losing rabbits from various complications by this method.

After three injections with the quantities and at the intervals stated above, a small amount of blood is taken from the rabbit on the fifth or sixth day after the last injection. This blood is then titrated in order to determine whether its hemolytic action is of sufficient strength for future work. If the blood serum is found to be of a sufficiently high titre, the rabbit may be either bled to death, or, what is far more satisfactory, about 15 c. c. of blood may be drawn from the veins of the ear. By the latter method the rabbit may be used continuously for the production of hemolytic serum. If it is desired to obtain the blood by bleeding the rabbit to death, the animal is anesthetized by a mixture of chloroform and ether, the hair on the neck is shaved, the skin is disinfected, and an incision made on one side of the neck in order to sever both the jugular vein and the carotid artery. Should the flow of blood cease on this side, the other side may also be cut. The blood is collected in centrifuge tubes, and after the bleeding has been completed centrifugalization of the blood is accomplished and the supernatant serum drawn off with a pipette.

Bleeding of the rabbit through the veins of the ear is best accomplished in the following way: After washing the ear and closely clipping the hair over the veins on the outside of the ear, the skin is disinfected with 75 per cent alcohol. Then a pledget of cotton is soaked in hot water (about 45° or 50° C.) and wound around the base of the ear in order to produce a hyperemia of the blood vessels. When a sufficient dilation of the vessels is observed, the middle and posterior auricular veins are severed, and the blood is then collected in centrifuge tubes. If the blood ceases to flow, the cotton should be removed from the base of the ear, and after placing it in hot water it is again applied to the ear and the hyperemia is thereby reestablished. In case the coagulation of the blood has prevented its flow at the place where the veins were severed, the wound may be scraped with a knife, and usually the blood will commence to flow freely again. The collected blood is then treated in the same manner as described above—that is, centrifuged—and the supernatant serum pipetted off.

Should this hemolytic rabbit serum be used before it is 3 days old, it must be inactivated by heating to 56° C. for one-half hour. After this time the hemolytic rabbit serum is preserved with one-half of 1 per cent of carbolic acid—that is, to each 9 c. c. of serum 1 c. c. of a 5 per cent carbolic-acid solution is added. Serum preserved in this way may be kept for two or three months. However, it should be retitrated every two or three weeks, as occasionally the titre of the serum drops. Such carbolized hemolytic serum does not require inactivation.

When preparing hemolytic serum it is best to start with several rabbits, as occasionally one may die as a result of anaphylaxis, and, again, some rabbits are not adapted for the production of hemolysins.

The titre of the hemolytic serum from a rabbit does not remain stationary, but for two or three weeks after the last injection it gradually lowers until it reaches that of a normal animal. If, however, such a rabbit is reinjected with washed corpuscles after several weeks, the hemolysins will again appear after a short time. Hemolysins are kept more or less in reserve in the cells of such an animal. The renewed injection acts as a stimulant, and these bodies are quickly thrown off into the blood, while in a normal animal they form slowly, first being formed in the cells. Thus, the advisability of keeping rabbits which have been bled from the veins of the ear for further production of hemolytic amboceptors can readily be seen.

TITRATION OF HEMOLYTIC RABBIT SERUM.

The hemolytic rabbit serum is titrated in order to establish the smallest quantity of serum that will produce hemolysis in the presence of a certain quantity of complement and the suspension of washed blood corpuscles of a sheep. The amount of hemolytic serum that will produce complete hemolysis in two hours at 37° C. is an amboceptor unit.

For the preliminary work of titration 10 test tubes are taken, and dilutions of the hemolytic serum are made with salt solution in proportions of 1 to 100, 200, 400, 500, etc., up to 4,000. These dilutions are made from basic dilutions of 1 to 100 and 1 to 1,000 as may be seen from Table 1.

Table 1.—*Titration of rabbit serum (hemolytic amboceptor).*

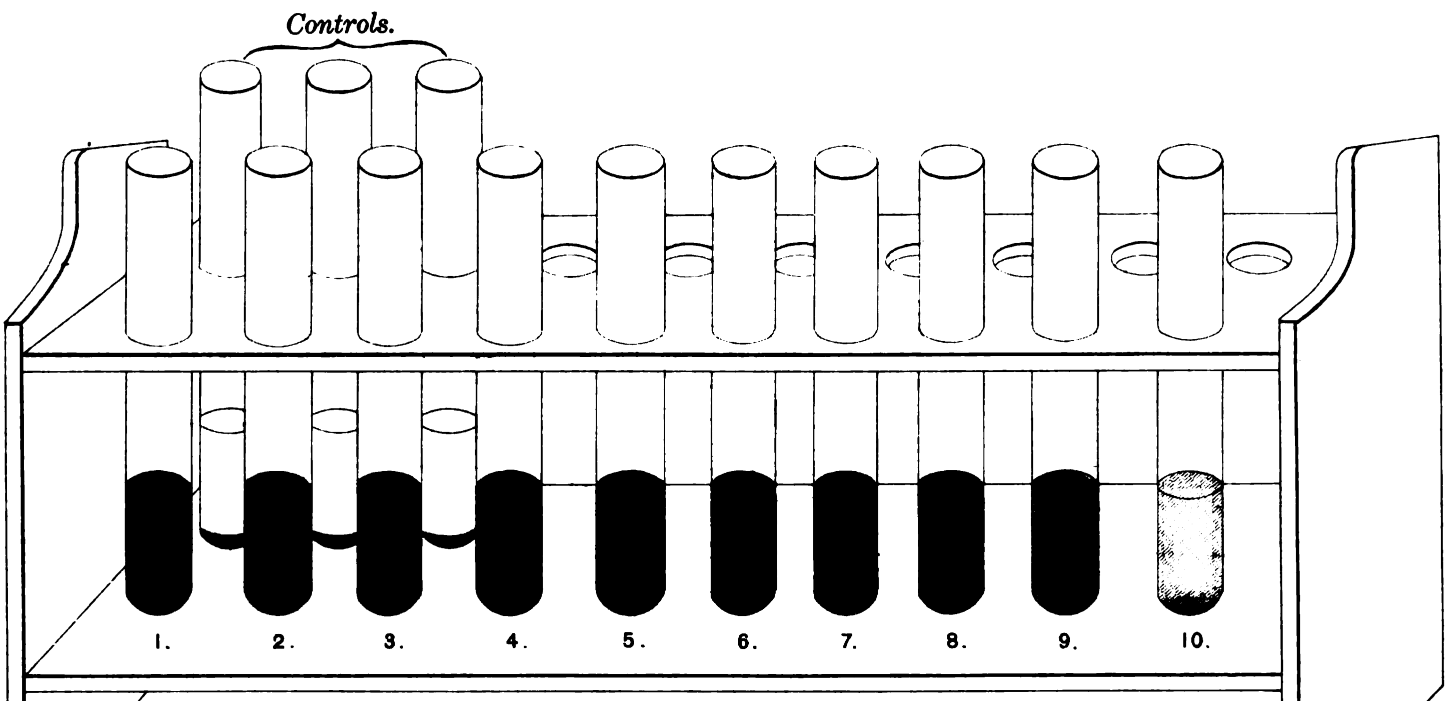
Tube.	NaCl solution.[1]	Amboceptor.	Complement.[2]	Blood corpuscles.[3]	Remarks.
	c. c.	1 c. c. of—	c. c.	c. c.	
1	2.5	1: 100	0.5	1	
2	2.5	1: 200	.5	1	
3	2.5	1: 400	.5	1	
4	2.5	1: 500	.5	1	
5	2.5	1: 600	.5	1	
6	2.5	1: 800	.5	1	
7	2.5	1:1,000	.5	1	
8	2.5	1:1,500	.5	1	
9	2.5	1:2,000	.5	1	
10	2.5	1:4,000	.5	1	
11	3.5		.5	1	Complement control (no hemolysis should occur).
12	3.0	1: 100		1	Amboceptor control (no hemolysis should occur).
13	4.0			1	Salt-solution control (no hemolysis should occur).

[1] 0.85 per cent NaCl solution.
[2] 0.5 c. c. of a 10 per cent solution of the complement.
[3] 5 per cent suspension of sheep-blood corpuscles washed in salt solution.

From each batch of serum taken from the rabbit a basic dilution is made in the proportion of 1 to 100, 200, 400, etc., up to 4,000. From these basic dilutions the third column in Table 1 is made, as indicated below:

A dilution of—
200 is made by adding 1 c. c. of 1 to 100 dilution to 1 c. c. NaCl solution.
400 is made by adding 1 c. c. of 1 to 100 dilution to 3 c. c. NaCl solution.
500 is made by adding 1 c. c. of 1 to 100 dilution to 4 c. c. NaCl solution.
600 is made by adding 1 c. c. of 1 to 100 dilution to 5 c. c. NaCl solution.
800 is made by adding 1 c. c. of 1 to 100 dilution to 7 c. c. NaCl solution.
1,000 is made by adding 1 c. c. of 1 to 100 dilution to 9 c. c. NaCl solution.
1,500 is made by adding 2 c. c. of 1 to 1,000 dilution to 1 c. c. NaCl solution.
2,000 is made by adding 1 c. c. of 1 to 1,000 dilution to 1 c. c. NaCl solution.
4,000 is made by adding 1 c. c. of 1 to 1,000 dilution to 3 c. c. NaCl solution.

It will be observed that the last three are made from the 1 to 1,000 dilution.



TITRATION OF HEMOLYTIC AMBOCEPTOR (RABBIT SERUM).

(Tube 8 is highest dilution showing hemolysis, and therefore represents the titre.)

THE [illegible] LITHO. CO. WASHINGTON D. C.

The titration proper is then made in the following manner: Ten additional test tubes are each filled with 2.5 c. c. of salt solution, to which is then added the hemolytic serum (amboceptor) in quantities of 1 c. c. of the different dilutions to each tube. Thus, in the first tube we add 1 c. c. of the dilution of 1 to 100; in the next, 1 c. c. of the dilution of 1 to 200; and in the third, 1 c. c. of the dilution of 1 to 400, etc. Afterwards the complement of the guinea-pig serum is added in quantities of 0.5 c. c. of a 10 per cent solution to each tube, and finally 1 c. c. of a 5 per cent suspension of sheep corpuscles in salt solution is placed in each tube.

Besides these 10 tubes, there are also three control tubes, one to show that the complement alone (without the amboceptor) will not produce hemolysis, the second that the amboceptor alone without the complement will not produce hemolysis, and the third that the salt solution alone will not produce hemolysis. Thus, in the first control tube we add 3.5 c. c. of salt solution, 0.5 c. c. complement, and 1 c. c. suspension of sheep corpuscles. In the second control tube we add 3 c. c. salt solution, 1 c. c. amboceptor of the 1 to 100 dilution, and 1 c. c. suspension of sheep corpuscles. In the third control tube we add 4 c. c. salt solution and 1 c. c. sheep corpuscles. It is advisable to place the tubes for the titration test in the lower row and the control tubes in the upper row of the test-tube rack.

As can be seen from the various quantities added to the tubes, the final volume is always uniformly 5 c. c. Thus, the different amounts of the blood derivatives used are always made up to 5 c. c. by the addition of salt solution.

After adding the substances in the test tubes in the order given, the tubes are well shaken, placed in a rack, and the rack put in an incubator at 37° C. for two hours. Then it is removed from the incubator and the results are read. (See Pl. II.)

The highest dilution in which complete hemolysis has taken place represents the titre of the hemolytic amboceptor. Thus, if complete hemolysis has taken place in the tubes where the dilution used in the rabbit serum was 1 to 2,000, the hemolytic amboceptor of that serum is represented by 2,000. This titre, however, is not used in the glanders test, but rather its double strength, which would be 1 to 1,000.

The titre of the hemolytic amboceptor for use in glanders diagnosis should not be less than 1 to 1,000, and therefore if the rabbit serum should prove of a lower titre it should not be used for this test. A low titre of the hemolytic amboceptor would disturb the results of the test, inasmuch as in such low dilutions too much serum would have to be used, and as the serum contains other substances in addition to amboceptors it would have an influencing effect on the hemolysis.

It is advisable to preserve the carbolized hemolytic amboceptor in small vials containing 1 to 2 c. c. of the rabbit serum, sealing them with paraffin. By this procedure the contents of a vial are used up more quickly and without frequent exposure.

The titration test of the hemolytic amboceptor is given in consecutive order in Table 1, and the method of carrying out the tests relative to the addition of the different substances is likewise given in consecutive order in all the tables. Thus the number of test tubes used is indicated, as well as the various substances and the quantities to be added, as they follow each other. It is our opinion that by this method the description of the tests can be followed without much difficulty, and by keeping these tables in the foreground the tests themselves may be applied readily, even by those who have had but little experience in this work. Of course accuracy in technique is the most important factor in the success of this line of serum diagnosis, and too much emphasis can not be laid upon this fact.

METHOD OF OBTAINING COMPLEMENT (GUINEA-PIG SERUM).

The complement represents the blood serum of a healthy guinea pig, and is obtained by bleeding the animal by severing the carotid and the jugular.

The guinea pig is anesthetized with a mixture of chloroform and ether, and the neck is shaved and then disinfected with a 75 per cent solution of alcohol. The animal is held by an assistant with its head down, while the operator uses his left hand to pull taut the skin over the region of the throat and his right hand to make an incision on one side of the neck by which the carotid and the jugular are severed. The centrifuge tube is immediately held under the opening, in order to collect all the blood. After the flow ceases, the same technique is practiced on the other side of the neck. Care should be taken to avoid the cutting of the trachea.

The blood thus collected, which usually amounts to 10 or 12 c. c., is placed in the ice chest, and after one or two hours the serum separates from the clot. The serum is then drawn off, and the rest is placed in a centrifugal machine, in order to obtain all the serum present in the clot. The guinea-pig serum should always be used fresh, and it is never advisable to use it after the second day, as the complement becomes considerably reduced upon standing.

TITRATION OF COMPLEMENT.

It is desirable to titrate the complement of every guinea pig, as such practice will insure more accurate work and better results in the glanders test. By titration of the complement we aim to establish the complement unit which is the smallest quantity of complement necessary to produce complete hemolysis in the presence of one amboceptor unit and a suspension of blood corpuscles of sheep. The smallest quantity is then taken as its test value. For the titration of the complement six test tubes are used, in addition to the three for controls. A 10 per cent basic dilution is made of the complement in the first test tube; that is, to 2.7 c. c. of salt solution 0.3 c. c. of complement is added. From this basic dilution the other dilutions are made by consecutively reducing the quantity of complement in the different test tubes. Thus, in the second test tube 0.5 c. c. of the 10 per cent basic dilution is added, in the third 0.4 c. c., in the fourth 0.3, and so on. Of course we add to each of these test tubes sufficient quantities of salt solution to make the required 3 c. c. Thus, into the second test tube we add 2.5 c. c., in the third 2.6 c. c., etc., of salt solution. After the complement has been added in quantities stated above, it is followed by the amboceptor. One cubic centimeter of the hemolytic amboceptor of which the titre has previously been determined is added, and finally 1 c. c. of a 5 per cent emulsion of blood corpuscles. The purpose of the controls is to establish in the first that the complement alone without the amboceptor does not produce hemolysis; in the second, that the amboceptor alone without the complement produces no hemolysis; and in the third, that the salt solution alone does not produce hemolysis. The first control tube contains 3.5 c. c. salt solution, 0.5 c. c. of the 10 per cent basic dilution, and 1 c. c. of suspension of corpuscles; in the second control 3 c. c. of salt solution, 1 c. c. of the amboceptor dilution, and 1 c. c. of suspension of blood corpuscles; in the third control, 4 c. c. of salt solution and 1 c. c. of suspension of blood corpuscles are used.

After shaking all the tubes, the rack is placed in the incubator for two hours, and removed in order to read the results. (See Pl. III.) The highest dilution of complement in the tube in which the hemolysis is complete indicates the titre of the complement. For instance, if hemolysis is complete in the tube where 0.3 c. c. of the 10 per cent basic dilution of the complement has been used, and hemolysis is incomplete in the tube in which 0.2 c. c. of a 10 per cent basic dilution has been used, then the titre of the complement is 0.03 c. c., inasmuch as we have started with a 10 per cent basic dilution. Thus, in the tests in this instance it would be necessary to use a 3 per cent complement solution.

In Table 2, the titration of the complement is given as in Table 1 for the hemolytic amboceptor, and this can be followed without much difficulty. Particular care, however, should be taken to use exact quantities as designated in the table.

TABLE 2.—*Titration of complement.*

Tube.	NaCl solution.[1]	Complement.[2]	Amboceptor.[3]	Blood corpuscles.[4]	Remarks.
	c. c.	c. c.	c. c.	c. c.	
1	2.7	0.3	....	....	Basic dilution of complement.
2	2.5	[5].5	1	1	Tubes 2 to 6 are for establishing the smallest quantity of complement which produces complete hemolysis. This smallest quantity is then taken as its test value. For instance, if smallest quantity is 0.03, then to 97 c. c. NaCl solution 3 c. c. complement is added; if it is 0.02, only 2 c. c. is added to 98 c. c. NaCl solution.
3	2.6	.4	1	1	
4	2.7	.3	1	1	
5	2.8	.2	1	1	
6	2.9	.1	1	1	
7	3.5	.5	....	1	Complement control (no hemolysis should occur).
8	3.0	....	1	1	Amboceptor control (no hemolysis should occur).
9	4.0	....	....	1	Salt-solution control (no hemolysis should occur).

[1] 0.85 per cent NaCl solution.
[2] Guinea pig serum in diminishing quantities.
[3] Of previously titrated hemolytic serum, double dissolving quantity.
[4] 5 per cent suspension of sheep blood corpuscles washed in salt solution.
[5] This amount is taken from the basic dilution in tube 1.

The amboceptor should be inactivated when used fresh; that is, before it is three days old. Otherwise it is carbolized with 10 per cent of 5 per cent carbolic-acid solution, and kept in ice box—in this case it is used without inactivation.

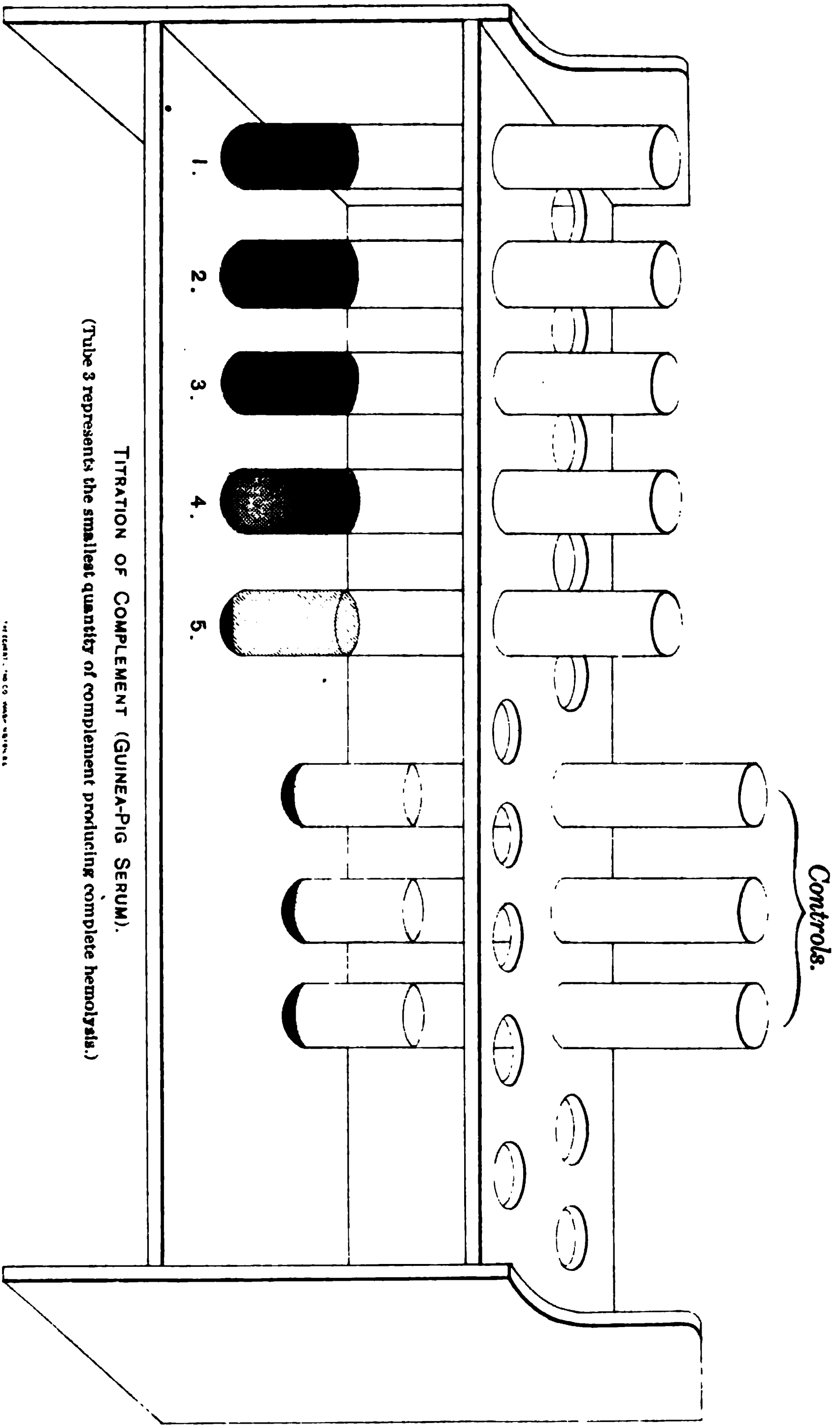
Place tube rack in incubator for 2 hours and read results.

This test should be made as a preliminary to every suspected glanders serum test, as it is always necessary to determine the smallest quantity of complement to be used for the final test. Of course, within 24 hours any number of tests can be made with the same complement dilutions.

SPECIFIC COMPLEMENT FIXATION (DEVIATION).

Complement fixation is a biological reaction in which the phenomenon of hemolysis is employed as the fundamental principle. It is so called on account of the fact that the complement has been fixed by the combination of antigen with antibody and thus prevented from participating in the hemolytic process in which it is essential in order to have hemolysis. By this method even small quantities of amboceptors (antibodies) can be demonstrated in a serum.

The presence of an infectious principle in the organism of an animal or a man has a stimulating effect on the production of antibodies (immune bodies). If a serum containing such immune bodies is inactivated and brought into contact with the antigen in the presence of complement, the complement will become firmly fixed by the combined immune body and antigen. (See Pl. I, B.) Thus, anchoring takes place between the antigen and the antibody in which the complement becomes fixed. This anchoring is thoroughly established when the mixture is placed in an incubator for one hour. The addition of the hemolytic amboceptor and blood corpuscles to



TITRATION OF COMPLEMENT (GUINEA-PIG SERUM).

(Tube 3 represents the smallest quantity of complement producing complete hemolysis.)

[illegible]

such an anchored antigen and immune body will have no effect. (See Pl I, D.) Thus, no hemolysis will take place, inasmuch as the complement has been fixed by the immune body and the antigen, thereby leaving the hemolytic system incomplete. On the other hand, if the inactivated serum contains no immune bodies, there would be no substance in the serum to anchor the antigen. As a result, therefore, no fixation of complement will occur, this being left free, and on addition of hemolytic amboceptor and blood corpuscles, hemolysis will now take place. (See Pl. I, C.) Neither the antigen nor the antibody alone can fix the complement and thereby influence hemolysis when the hemolytic amboceptor and blood corpuscles are added. However, in combination the fixation will invariably take place, and on the addition of the hemolytic amboceptor and blood corpuscles hemolysis will not be produced.

Since the discovery of this phenomenon it has been utilized extensively in serum diagnosis, but probably its greatest value has been obtained from the Wassermann reaction for the diagnosis of syphilis. It has also been employed in other diseases with more or less satisfaction, and its great field in bacteriological investigations has not yet been exhausted for the practical diagnosis and determination of immune bodies in serum. In veterinary practice complement fixation is now gradually becoming used for the diagnosis of glanders. This method of diagnosing glanders has given the most favorable results in Germany, and constitutes at the present time the official test for Prussia and other parts of Germany. It has also been used in the diagnosis of other diseases of animals, but not with such success as in glanders. Particularly in tuberculosis the results were not uniform and otherwise not very promising.

The presence of the specific immune bodies (bacteriolytic amboceptors) in the serum of glandered horses brings about the fixation of the complement when the antigen in the form of glanders bacilli extract is added to the hemolytic system. The serum of glandered horses, therefore, contains antibodies (immune bodies) against glanders bacilli, which are specific only for the glanders bacilli and for no other infection. The complement fixation accordingly represents a specific test, as only in the presence of the glanderous immune bodies and glanderous antigen will the reaction take place. If, instead of the glanderous immune bodies, other antibodies of another infectious disease be present in the blood serum, they will exert no effect whatsoever on the glanderous antigen; and, on the other hand, if serum containing glanderous immune bodies is brought in contact with an antigen of another infectious disease, it will also have no effect on the reaction. By this fixation of the complement the hemolytic system is left incomplete, and as a result no hemolysis will take place. This fixation of the complement by the antigen and immune bodies of glanders in the horse serum constitutes the diagnostic test for this disease.

In the application of the test it is necessary to have substances constituting the hemolytic system, which are the washed blood corpuscles of a sheep, the hemolytic amboceptor (rabbit serum), and complement (normal guinea pig serum). The quantity of the hemolytic amboceptor to be used has been established by titration and described in a preceding part of this publication. In the test for glanders the double strength of the determined titre of the hemolytic amboceptor is used. A slight excess in the quantity of the amboceptor does not alter the outcome of the tests.

On the other hand, the establishment of the smallest amount of complement which will produce hemolysis in the presence of the hemolytic amboceptor and blood corpuscles is very essential, and accordingly this should be established by the previously described preliminary test, before tests for glanders are undertaken. By omitting this it is possible that an excessive amount of complement would be used which would in some cases affect the final results of the test. An oversupply of complement in the test may not only prove sufficient to be fixed by the immune bodies (antibodies) and antigen, but there might be also enough complement left to produce an incomplete or an almost complete hemolysis. Thus it is evident that it is of great importance to establish by such a preliminary test (see Table 2) the exact quantity of complement to be used. Schütz and Schubert found that the success of this method of diagnosing glanders depended greatly upon the proper quantity of complement used, and therefore the establishment of this quantity should not be omitted.

The red blood corpuscles of the hemolytic system always constitute a uniform quantity—that is, a 5 per cent suspension of the washed corpuscles in salt solution. As has been stated, this may be kept for testing purposes for two or even three days in the ice chest. The method of obtaining the corpuscles has been described in the early part of this work.

In the complement-fixation test, there are also used, besides the hemolytic system, the serum of the horse to be examined and antigen.

METHOD OF OBTAINING SERUM OF ANIMALS TO BE TESTED.

The blood is drawn from the jugular vein of the suspected horse after a small area over the jugular has been clipped and disinfected with alcohol. The vein is dilated by pressure on the lower part of the neck, and the blood is drawn from the animal by the insertion of a trocar and cannula. It is recommended that the blood should be collected in uniform-sized tubes or bottles, which should be sterilized before using. A sufficient quantity of blood for testing purposes would be 50 to 100 c. c., and after allowing the blood serum in the tube to separate from the clot in a cool, dark place it is ready to be used for the test. If it is desired to forward the blood to a laboratory the tubes may be packed into separate containers or collectively in a box. Every tube should be labeled, and the number of the horse corresponding with the record number should be designated on the label. It is not absolutely essential to have clear serum, as in repeated tests carried out in this laboratory it was found that blood forwarded from Michigan to Washington gave satisfactory reactions, although the serum was badly discolored as a result of disintegration of the blood corpuscles. If it is desired to preserve the serum, or if from some cause a test can not be applied during the first few days after the blood has been drawn, it should be preserved with a 0.5 per cent solution of carbolic acid. This percentage is best obtained by adding 1 part of a 5 per cent carbolic-acid solution to 9 parts of the serum to be preserved. Such carbolized serum will respond to this test after several months.

Of the serum to be examined about 2 c. c. is drawn off and placed in a suitable tube or bottle in order to subject it to a temperature of 58° C. in a water bath for one-half hour. This constitutes the inactivation of the serum; that is, the complement which is present in the serum is destroyed by this heating. Such inactivated serum is ready for use in the testing, but should be used only on the day of its inactivation. In case it becomes necessary to repeat the test, another 2 c. c. of the sample should be inactivated.

In cases where the mallein test has been used the blood of suspected horses to be examined for glanders by complement fixation should not be taken until from 7 to 10 days have elapsed after the last mallein test. This is necessary because of the possibility that the injected mallein may have exerted a stimulating effect on the cells with the production of immune bodies, and if serum is then taken for the test the results may lead to a faulty diagnosis. However, in suspected cases of glanders the blood of the animals may be drawn and forwarded for examination to a laboratory where the serum diagnosis of glanders is practiced, without the necessity of the application of the mallein test.

PREPARATION OF THE ANTIGEN (GLANDERS BACILLI EXTRACT).

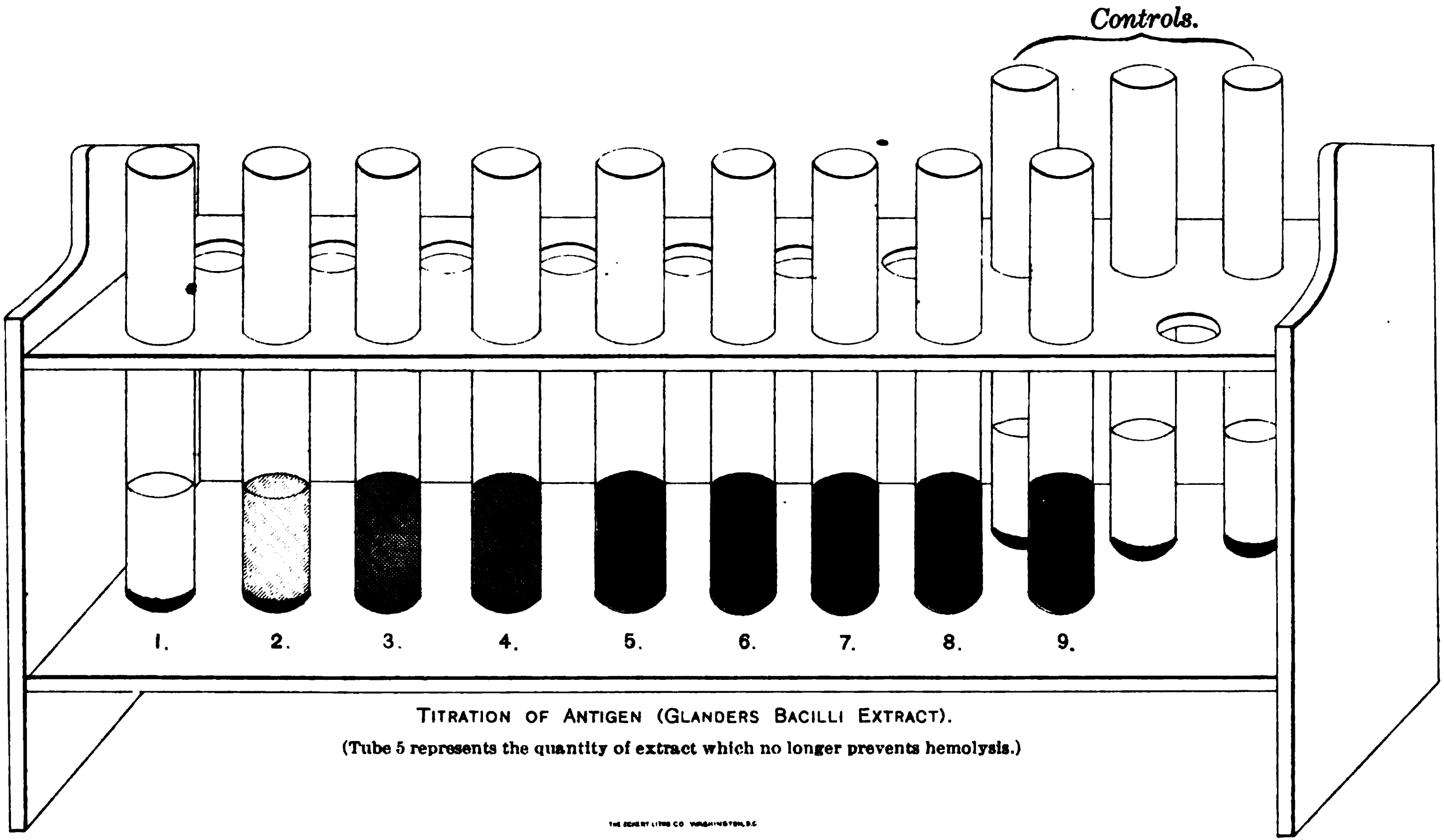
The antigen represents a shake extract of glanders bacilli in salt solution. It is prepared as follows: From a stock culture of glanders bacilli subcultures are made on 2 per cent acid glycerin agar media. It is preferable to use Kolle flasks instead of tubes for the cultures, inasmuch as in such flasks the surface of the media is much larger and a greater quantity of bacilli can be obtained from them. After inoculating the media with glanders bacilli the flasks are placed in the incubator, and after 24 hours the condensation water in the cultures is allowed to run over the surface of the media. After another 24 or 48 hours in the incubator the surface of the media contains usually a luxuriant growth of glanders bacilli, and it is then ready for washing. To each flask 20 to 40 c. c. of salt solution is added. If the cultures have been grown in ordinary test tubes they are washed off with 5 to 15 c. c. of salt solution.

After the entire growth is washed off the surface of the medium the fluid is poured into sterile flasks and then heated to 60° C. for four hours in order to kill the glanders bacilli. After the heating of the bacilli the flasks are placed in a shaking apparatus and shaken for four days.

After removal of the flasks the extract is placed in centrifuge tubes and centrifugalized for two hours in a centrifuge at a speed of 2,500 to 3,000 revolutions per minute. Then to the clear liquid, which has been drawn off into suitable bottles, 10 per cent of a 5 per cent carbolic-acid solution is added, and the bottles are corked. This represents the extract or antigen to be used for the tests in a dilution which is established by titration. The extract usually keeps for two or three months, or sometimes even longer if kept in a dark, cool place.

TITRATION OF THE EXTRACT.

The titration of the extract is carried out in order to establish the quantity of the extract which no longer prevents hemolysis. First, dilutions with salt solution are made from the extract in proportions of 1 to 20, 30, 40, 50, and so on up to 200. These dilutions are made from a basic dilution of 1 to 10, as can be seen from Table 3. The titration proper is carried out as follows: Nine test tubes are used for the titration and three for controls. To each tube 1 c. c. of salt solution is added, then 1 c. c. of complement of the determined smallest quantity established according to the preliminary test (see Table 2). This is followed by the extract, each tube receiving 1 c. c. of the different dilutions prepared. Thus, in the first tube 1 c. c. of the dilution of 1 to 10 is added; in the second, 1 c. c. of the dilution of 1 to 20; in the third, 1 c. c. of the dilution of 1 to 30, and so on. Then the rack is placed in the incubator for one hour. After it is taken out, 1 c. c. of a double quantity of the previously titrated amboceptor is added to each tube, and finally 1 c. c. of a 5 per cent suspension of washed sheep blood corpuscles. Of the three control tubes, the first serves for a control to show that the complement alone (without the amboceptor) does not produce hemolysis, the second to show that the amboceptor alone does not produce hemolysis, and the third that the salt solution alone does not produce hemolysis. Thus, in the first control tube 3 c. c. of salt solution, 1 c. c. of complement,



TITRATION OF ANTIGEN (GLANDERS BACILLI EXTRACT).

(Tube 5 represents the quantity of extract which no longer prevents hemolysis.)

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and 1 c. c. of blood corpuscles are added. The second tube contains 3 c. c. of salt solution, 1 c. c. of amboceptor, and 1 c. c. of blood corpuscles, while the third contains 4 c. c. of salt solution and 1 c. c. of blood corpuscles. This titration is similar to the titration of the hemolytic amboceptor.

After shaking the test tubes, which should invariably follow all these additions, the rack is placed in the incubator for two hours. Then the results are read. The tube in which hemolysis is no longer prevented represents the titre of the extract. (See Pl. IV.) In the glanders test, however, one-half of that quantity is used. Thus, if the titration should prove that the first tube which does not prevent hemolysis contains a dilution of 1 to 50, then a dilution of 1 to 100 of the extract is used for the glanders tests.

In Table 3 the titration of the glanders bacilli extract is given in consecutive order, and this may be followed in a similar manner as with the other tables. In this table the substances to be used are given consecutively, and likewise the exact quantities are indicated.

TABLE 3.—*Titration of glanders bacilli extract.*

Tube.	NaCl solution.[1]	Complement.[2]	Extract.[3]		Amboceptor.[4]	Blood corpuscles.[5]	Remarks.
	c. c.	*c. c.*	1 c. c. of—		*c. c.*	*c. c.*	
1	1	1	1 : 10...	Place for 1 hour in incubator.	1	1	One-half of the quantity of the extract which no longer prevents hemolysis is the proper value to be used in future final testing. Thus, if it should show by this test to be 1 to 50, then a dilution of 1 to 100 of the extract is used.
2	1	1	1 : 20...		1	1	
3	1	1	1 : 30...		1	1	
4	1	1	1 : 40...		1	1	
5	1	1	1 : 50...		1	1	
6	1	1	1 : 60...		1	1	
7	1	1	1 : 80...		1	1	
8	1	1	1 : 100..		1	1	
9	1	1	1 : 200..		1	1	
10	3	1	....		....	1	Complement control (no hemolysis should occur).
11	3	....	....		1	1	Amboceptor control (no hemolysis should occur).
12	4	....	....		....	1	Salt solution control (no hemolysis should occur).

[1] 0.85 per cent NaCl solution.

[2] The determined smallest quantity established according to the preliminary test.

[3] A basic dilution is made from the extract in proportions of 1 to 10, 20, 30, etc., up to 200. From these basic dilutions the third column is made as indicated below.

[4] Double the quantity previously determined by titration.

[5] 5 per cent suspension of sheep-blood corpuscles washed in salt solution.

The basic dilutions of the glanders bacilli extract are made as follows:

A dilution of—
1 to 20 is made by adding 1 c. c. of 1 to 10 dilution to 1 c. c. NaCl solution.
1 to 30 is made by adding 1 c. c. of 1 to 10 dilution to 2 c. c. NaCl solution.
1 to 40 is made by adding 1 c. c. of 1 to 10 dilution to 3 c. c. NaCl solution.
1 to 50 is made by adding 1 c. c. of 1 to 10 dilution to 4 c. c. NaCl solution.
1 to 60 is made by adding 1 c. c. of 1 to 10 dilution to 5 c. c. NaCl solution.
1 to 80 is made by adding 1 c. c. of 1 to 10 dilution to 7 c. c. NaCl solution.
1 to 100 is made by adding 1 c. c. of 1 to 10 dilution to 9 c. c. NaCl solution.
1 to 200 is made by adding 1 c. c. of 1 to 100 dilution to 1 c. c. NaCl solution.

THE COMPLEMENT FIXATION TEST.

As previously stated, the serum of glandered horses contains immune bodies which develop during the course of the disease. The presence of these immune bodies in the serum is utilized in the development of the test. Naturally, the amount of these immune bodies in the affected horses is not uniform and undoubtedly depends on the extent of the infection present; therefore it is advisable to use in the test such quantities of the serum as will prove sufficient for the reaction to take place.

The necessary quantity for the test has accordingly been established, through the painstaking experiments of Shütz and Schubert, as 0.2 and 0.1 c. c., placed in two different tubes. In some instances the tube containing 0.2 c. c. of the serum may show a fixation of the complement, while the tube containing 0.1 c. c. of the same serum may show only a partial fixation or hemolysis. In the great majority of cases, however, the fixation is usually manifested in both tubes.

The substances which are used in the fixation test have already been described in detail, their strength has been established by the titration of these different substances, and it need only be mentioned in passing that the best results will be obtained when the smallest quantities of substances permitted by the titration are used for the test; that is, the substances should have a high titre. If, for instance, the glanders extract should prove of a low titre, the albumins which are contained therein may have an influencing effect upon the outcome of the test, inasmuch as it is known that albumins or cell extracts, if present in large quantities, will fix complements. Therefore it is always advisable to use glanders extract of a high titre; the quantity to be used should not be much above 0.01 c. c.

Besides the centrifugal machine and an ordinary shaking apparatus, only glassware is needed for the test. This consists principally of pipettes of various sizes, and there should always be a sufficient number on hand during the performance of the test. Those best adapted for the work are 1 c. c. pipettes, graduated into hundredths, and 10 c. c. pipettes, graduated into tenths. In addition, several pipettes of various sizes will be found useful for measuring the various substances to be diluted. Uniform-sized test tubes are advised and they should fit into test-tube racks. All the glassware should be cleaned and dry-sterilized before using. Only such a number of tests as can be conveniently handled should be undertaken on a single day. Finally, the incubator should contain sufficient space to hold the necessary number of racks during the test. With a good-sized incubator and the above-mentioned apparatus any State bacteriological laboratory could meet the demand for diagnoses from all the veterinarians within its borders, as from 80 to 100 tests may readily be made by this method in one day.

APPLICATION OF THE TEST.

Before undertaking the tests it is advisable to prepare the dilutions which will be used for the testing. The quantities of dilutions can be determined quite accurately, in accordance with the number of tests to be made. Thus the necessary quantity of a suspension of blood corpuscles is prepared, as well as the dilution of the necessary quantity of the amboceptor, complement, and glanders extract. The preparation of these dilutions is best accomplished in Erlenmeyer flasks, and the dilutions prepared should not be kept over one day.

Four test tubes are used in testing the serum of each horse. For convenience in reading the results they are placed in a test-tube rack. The first test tube is marked with the number of the animal corresponding with that on the record for identification. Thus, for instance, should it be necessary to examine 20 horses, 80 tubes would be required for the tests. The first and third tubes are intended for the test proper, while the second and fourth tubes serve as controls for the horse serum to be examined. All the test tubes are first filled with the necessary quantity of salt solution, which will make up with the other substances used the required 5 c. c. Thus the first and third tubes each contain 1 c. c. of salt solution, while the second and fourth tubes contain 2 c. c. each. Then the suspected horse serum to be examined, which has been previously inactivated by heating for one-half hour at 58° C., is added. In the first and second tubes 0.1 c. c. and in the third and fourth tubes 0.2 c. c. of this inactivated serum is added. This is followed by the addition of the glanders bacilli extract, which, however, is only introduced into the first and third tubes, 1 c. c. of the established dilution being used. The second and fourth tubes, therefore, do not contain glanders bacilli extract, as these tubes serve only as controls to show that the horse serum alone will not influence hemolysis. Thus in every test the amount of serum used in the tests proper (first and third tubes) is controlled by an equal amount of serum (second and fourth tubes).

After the addition of the antigen (glanders extract), 1 c. c. of the complement is added to every tube in a dilution which has been established by the preliminary tests. (See Table 2.)

At this stage of the testing each tube contains 3 c. c. of fluid. The rack containing the tubes is now shaken and placed in the incubator for one hour. This incubation is carried out in order to allow the anchoring of the antigen and the glanders immune body, in which the complement will become firmly fixed. Naturally, the anchoring would only take place provided the serum contains the glanders immune bodies; that is, when the horse is infected with glanders. (See Pl. I, D.) On the other hand, in the absence of a glanders infection the serum does not contain immune bodies, and therefore the antigen as well as the complement will remain free in the test tubes. (See Pl. I, C.)

After the time required for the incubation has elapsed the rack is removed and the other substances of the hemolytic system are added, namely, the hemolytic amboceptor and the blood-corpuscles suspension.

To each test tube is added 1 c. c. of the previously titrated rabbit serum (hemolytic amboceptor), and finally 1 c. c. of a 5 per cent suspension of sheep blood corpuscles which have been previously washed with salt solution. The tubes, either individually, or collectively in the rack, should then be well shaken by hand, in order to mix the fluids thoroughly and to wash down any flecks of the solutions that might have adhered to the sides of the tubes.

This practically concludes the test, and it is now only necessary to incubate the tubes for 10 hours, when the results may be read.

CONTROLS.

During the application of all tests control tubes are also used at the same time for comparative purposes. Four tubes are required for the control of the hemolytic system and two for the control of the glanders extract. The control of the hemolytic system is established by using in the first tube all substances constituting the hemolytic system, namely, the hemolytic amboceptor, the complement, and the suspension of blood corpuscles. This tube after the conclusion of the test should show a complete hemolysis. In the second tube the hemolytic amboceptor is controlled without the complement, to prove that it does not produce hemolysis. The third tube serves as a control of the complement without the hemolytic system, also to prove that it does not produce hemolysis. The fourth tube is to show that the salt solution has no hemolytic action on the blood corpuscles. The quantities of the different substances used in the controls are the same as used in the regular tests for glanders. It is necessary in the control tubes, as well as in all other tubes, to make up the quantity to 5 c. c. with salt solution. Thus, in the tube which serves for the control of the hemolytic system, 2 c. c. of salt solution is added, while in the control of the hemolytic amboceptor as well as in the control of the complement 3 c. c., and in the salt-solution control 4 c. c., are added. The substances added to the control tubes are used in the same order as given in the test, and the incubation is also followed at the same time.

As above stated, two test tubes are used for the control of the antigen (glanders bacilli extract), in addition to the four used for the control of the hemolytic system. These two tubes contain the hemolytic system, and to the first tube 1 c. c. of the extract used in the glanders test is added, while to the second tube is added 2 c. c. of the extract. The controls of the extract serve to prove that the extract used in the tests does not cause a fixation of the complement, and that even double the quantity of the extract which has been used in the glanders test does not fix the complement. In the extract controls we have in the first 1 c. c. of salt solution, while to the second tube no salt solution is added, inasmuch as the substances in this control make up the necessary 5 c. c. In the first tube, besides the 1 c. c. of salt solution, 1 c. c. of the extract is added, which is followed in consecutive order with the substances of the hemolytic system, in quantities and dilutions used during the test proper, which have been previously indicated.

The results in the control tubes after the conclusion of the tests should show the following:

All the control tubes which contain, besides the hemolytic system, only serum of the suspected horse—that is, the second and fourth tubes—should show a complete or almost complete hemolysis. There should be no deposit of blood corpuscles on the bottom of these tubes, or only a very small quantity. The fluid should have the characteristic (laked) wine-red color.

In the control tubes of the extract there should also be a complete or almost complete hemolysis. The same should be present in the control of the hemolytic system; it should show a complete hemolysis. In the control tubes of the complement the hemolytic ambocepter, and the salt solution, there should be no hemolysis whatever; that is, the blood corpulscles should be settled in the bottom of the tube, and the liquid above them should be water-clear.

In Table 4 the final test for glanders is given in the order in which it should be undertaken. The number of test tubes to be used is indicated, and also the number of controls to be made. The substances and quantities, as well as the dilutions, are also indicated in the explanatory remarks of the table. As already stated, six controls are sufficient for any number of tests undertaken on one day, if the same substances are used for all the tests. In making the complement-fixation test it is always advisable to control the results further by using serum from an established case of glanders in one series of four tubes, and from a healthy horse in another similar series, for comparative purposes.

TABLE 4.—*The final test for glanders.*

Tube.	NaCl solution.[1]	Suspected horse serum.[2]	Glanders bacilli extract.[3]	Complement.[4]		Amboceptor.[5]	Blood corpuscles.[6]	Remarks.
	c. c.	*c. c.*	*c. c.*	*c. c.*		*c. c.*	*c. c.*	
1	1	0.1	1	1	Place for 1 hour in incubator.	1	1	Test tube for the dose 0.1 c. c. of suspected serum.
2	2	.1	....	1		1	1	Serum control for the dose 0.1 c. c. of suspected serum.
3	1	.2	1	1		1	1	Test tube for the dose 0.2 c. c. of suspected serum.
4	2	.2	....	1		1	1	Serum control for the dose 0.2 c. c. of suspected serum.
5	1	....	1	1		1	1	Control for the quantity of extract used (hemolysis).
6	....	....	2	1		1	1	Control for the double quantity of extract, for greater accuracy (hemolysis).
7	2	....	....	1		1	1	Control of the hemolytic system (hemolysis).
8	3	....	....	1		....	1	Control of the complement (no hemolysis).
9	3	....	....	....		1	1	Control of amboceptor (no hemolysis).
10	4	....	....	....		....	1	Control of salt solution (no hemolysis).

[1] 0.85 per cent NaCl solution.

[2] Suspected horse serum to be inactivated for 30 minutes at 58° C. in water bath, in order to destroy the complement which is present in the serum of every horse.

[3] One-half of the quantity which does not prevent hemolysis and established by titration. (See Table 3.)

[4] The determined smallest quantity established according to the preliminary test. (See Table 2.)

[5] Double the quantity previously determined by titration. (See Table 1.)

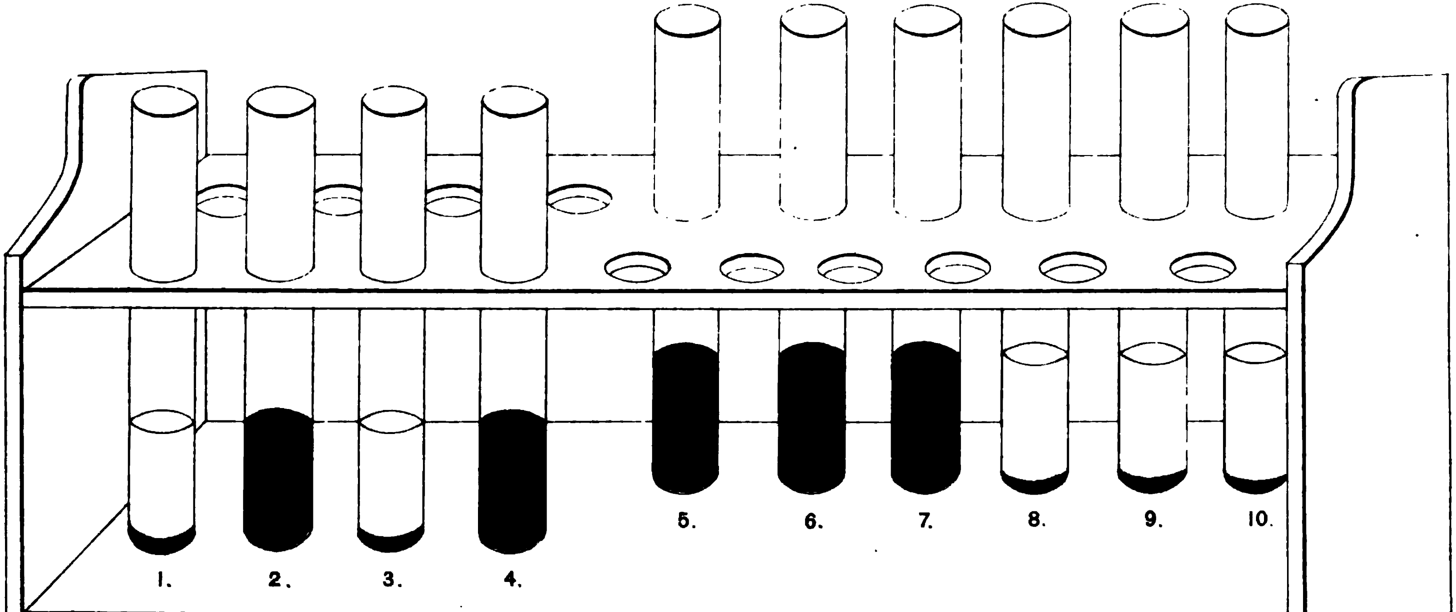
[6] 5 per cent suspension of washed sheep-blood corpuscles.

INTERPRETING RESULTS OF TESTS.

The results of the tests are manifested in most instances by a distinct reaction which takes place in the test tubes. The indication of the results is only to be sought in the tubes numbered 1 and 3, which represent the actual tests for the presence or absence of glanders, inasmuch as these tubes contain all substances required in the tests.

We may thus obtain in these tubes either complete hemolysis, incomplete hemolysis, or no hemolysis whatsoever. The fixation of the complement is manifested by the absence of hemolysis, and therefore we have in the first and third tubes a settling of the blood corpuscles with the watery clear fluid above. Such a result indicates without doubt the presence of glanders. On the other hand, if the first and third tubes show complete hemolysis, the absence of glanders is thereby indicated. In the presence of glanders a fixation of the complement takes place, as a result of anchoring to the immune bodies and antigen (see Pl. I, D), while in the absence of glanders, there being no immune bodies present, the complement is used up in the phenomenon of hemolysis (see Pl. I, C).

Then, again, we may have cases in which the fixation of the complement is incomplete; that is, there is a settling of corpuscles in the bottom of the test tube, but the fluid shows traces of hemolysis. It



FINAL TEST SHOWING POSITIVE REACTION TO GLANDERS.

Tubes 1 and 3 show fixation of complement indicative of glanders.
Tubes 2 and 4, controls for the suspected horse serum.
Tubes 5 and 6, controls for the antigen.
Tube 7, control for hemolytic system.
Tube 8, control for complement.
Tube 9, control for hemolytic amboceptor.
Tube 10, control for salt solution.

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does not show the characteristic saturated color of hemolysis, but only a tingeing with the hemoglobin. This is termed an almost complete fixation, and also indicates the presence of glanders. The presence of the characteristic color in the fluid and a very slight deposit of corpuscles on the bottom should not be taken as an indication of an infection, as such a condition may be brought on by various causes, and particularly so by the presence of nonspecific substances in the serum of the horse, which may cause a very slight checking of the hemolysis. But all cases where the results show a fixation of the complement (no hemolysis) or almost complete fixation (slight tingeing of the fluid above the settled blood corpuscles) indicate the presence of glanders.

As a result of the different quantities of horse serum used in the two pairs of test tubes, it will be found occasionally that the test tube containing the larger quantity of serum (0.2 c. c.—third tube) shows a fixation of the complement, while the tube containing the smaller quantity of serum (0.1 c. c.—first tube) shows a partial fixation of the complement. This also can be considered as a glanderous infection, as it shows that there are present immune bodies, but not to such an extent as to produce the fixation of the complement in the tube where the smaller quantity of the suspected horse serum has been used. In most instances of glanderous infections, however, it will be observed that the fixation of the complement is uniform and almost complete in both tubes.

The reading should be made after the results in the test tube have been clearly established, and one should not be too hasty in drawing conclusions before the reaction in the test tubes is clear enough to establish the results of the test.

In the illustrations in Plate V the results of the glanders tests are indicated by the presence of a positive reaction. In tubes 1 and 3 a complete fixation of the complement has taken place, there being no hemolysis, while tubes 2 and 4 represent the controls for the horse serum (no glanders extract being added), these tubes showing hemolysis. In the upper row of the rack the controls are indicated, and they show the results as they should be obtained in the course of testing.

Should the horse prove glandered on the above test, the test may be repeated, and then serum quantities are used as follows: 0.1, 0.05, 0.03, 0.02, and 0.01 c. c. One control is made of 0.1 c. c. as in tube 2 of the test. By this method the smallest quantity of serum is established which will divert the complement, but it is not necessary as routine practice.

Our own experience, as well as the work of Schütz and Schubert, to whom we wish to extend our acknowledgments, shows that the results of the complement-fixation test should be interpreted as follows:

1. Horses in which the serum produces a complete fixation of the complement in the quantities of 0.1 c. c. and 0.2 c. c. should be considered as glandered.

2. Horses in which the serum gives a complete fixation in the quantity of 0.2 c. c. and an incomplete fixation in the quantity of 0.1 c. c. should likewise be considered as glandered.

3. Horses in which the serum produces an incomplete fixation of the complement in the quantities of 0.1 c. c. and 0.2 c. c. should also be considered as glandered.

4. Horses in which the serum shows no fixation of the complement in either tube should be considered free of glanders.

In order to reduce the possibility of error to a minimum the agglutination test may be applied to the latter cases, and if this shows a value of 1 to 1,000 or over, the animal should be considered as glandered. However, such cases are extremely rare.

The agglutination test may be undertaken with the complement-fixation test without a great deal of difficulty, especially if the agglutination test is carried out in accordance with the method used at the present time in Germany, by which the results of the test can be obtained in about two hours. This consists of a modification of the agglutination test, in which by centrifugalization the agglutination is hastened in the test tubes and the results can be read after the tubes have been placed in the incubator for two hours.

The technique of this method of agglutination testing will not be explained here, but it is planned to describe it in a later publication on this subject.

CONTROLLING GLANDERS IN AN INFECTED STABLE.

When glanders is discovered or suspected among the horses in a stable, the blood of all the horses in the infected stable should be drawn and tested in the manner previously described. All animals whose serum shows a complement fixation should be destroyed without further consideration. After the animals have been killed the stable should be thoroughly cleaned and disinfected. The animals which gave no reaction on the first test should be retested after three weeks, and should there be no indication of the disease in the second test the stable may be considered free from the infection. On the other hand, if on the second test one or more animals should give a reaction, the infected animals should be destroyed and all the remaining horses should again be subjected to another retest after three weeks. Not until the last test proves that no animal is affected can the stable be considered as free from the infection.

RESULTS OF PRACTICAL TESTS WITH COMPLEMENT FIXATION.

In order to ascertain how long a time after infection with glanders elapses before the presence of the disease could be detected by the complement-fixation test, a horse was infected with glanders at the Bureau of Animal Industry Experiment Station by taking a loopful of glanders culture and rubbing it up and down the Schneiderian membrane. Twenty-four hours after the infection the first sample of blood was taken from the jugular, and this was followed by taking samples daily for about a month. The horse was inoculated on January 17, and the blood which was taken on January 22 gave a positive reaction to the complement-fixation test, thereby proving that five days after infection the presence of glanders could be ascertained by complement fixation. The animal at that time showed no clinical indication of the disease, but three days later (on the eighth day) the discharge from the nose appeared, and at the same time the characteristic swelling of the submaxillary lymph glands was noted. Repeated tests made with blood taken daily from the horse gave always the characteristic reaction. After four weeks from the time of the infection the reaction appeared to be less pronounced, this probably being due to the acuteness of the disease and the great amount of toxin which had been produced continuously in the animal. The testing of this horse was not continued after the disease had affected the animal to such an extent that it appeared dangerous to continue the work, owing to the extensive infection accompanied by a very profuse discharge.

Since this method of diagnosis for glanders was inaugurated in this laboratory, large numbers of horses and mules have been examined in the District of Columbia, as well as animals from other parts of the United States. Many of the horses examined had clinical cases of glanders, while others were selected because they were reactors to the mallein test, some typically, and others atypically. A large proportion of the cases, however, were exposed or "contact" horses. From the number of tests already made—about 800—the results indicate that in the complement fixation we have a method which in accuracy is equal to the tuberculin test for the diagnosis of tuberculosis in cattle. The results of the tests thus far conducted show that at least 97 per cent of the cases of glanderous affections can be determined by the complement-fixation method.. Furthermore, the affected horses in which a negative or atypical reaction occurs are as a rule either very old chronic cases of glanders, or those fresh cases of infection tested during the period of incubation. According to Hutyra and Marek,[1] the diagnosis of glanders by the complement-fixation test has already given such accurate results that it may be considered as the best method for the determination of this disease at the present time.

A good opportunity was recently afforded to apply the test extensively in an outbreak of glanders which occurred during the month of January, 1911, in a fashionable boarding stable in the District of

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[1] Spezielle Pathologie und Therapie der Haustiere. Third edition, Band 1, 1910, p. 717.

Columbia. At this stable two clinical cases of glanders were found, and the physical examination of 60 horses in the stable showed three additional cases with not typical but somewhat suspicious symptoms. The testing of all the horses in the stable was immediately undertaken, and as a result of the complement-fixation tests 14 horses were found to be affected with glanders. Unfortunately it was impossible to obtain the consent of the proprietor to test the horses with mallein for a comparative study of these two methods of diagnosis.

Careful post-mortem examinations were made on all the reacting animals, and in every instance lesions of glanders were found in one or more organs or tissues. In some of the cases only a few characteristic pulmonary nodules were present, while in others the characteristic lesions of acute glanders of the lungs and liver were present. There was not a single case among these horses in which the diagnosis on post-mortem examination could have been doubted.

After removing and killing the affected animals the stable was thoroughly cleaned and disinfected, and a second test was made with the blood of the remaining horses three weeks from the time the first test had been conducted. On the second test all the horses except one gave an absolutely negative reaction. This horse was destroyed at once, and the post-mortem examination revealed a caseated focus in the left bronchial lymph gland and about twenty small nodules of glanders in the lungs, all of which were evidently recent, having developed without doubt during the interval between the tests, as a result of the previous heavy exposure.

In all other cases where the test has been applied and in which careful post-mortem examinations were held after the destruction of the positive cases the presence of glanders has been demonstrated. Of course not all the animals which gave a reaction were autopsied, inasmuch as in some instances the tests were made for State officials, the animals not being under the jurisdiction of the Department of Agriculture. Again, in other cases where the animals were destroyed, no data of the post-mortem examinations were furnished.

Among the horses tested by the complement fixation there were a number of animals which gave an atypical reaction to the mallein test, but on the complement-fixation test they proved either absolutely positive or negative. Of these horses those which gave a positive reaction and were killed proved to be glandered.

Table 5 shows the comparative results obtained with the mallein and complement-fixation tests:

Table 5.—*Comparative results with mallein and complement-fixation tests.*

[+ denotes positive reactions; – denotes negative reactions; ? denotes atypical reactions.]

Series.	Number of animals.	Mallein.	Complement fixation.	Mallein.	Complement fixation.	Mallein.	Complement fixation.	Mallein.	Complement fixation.	Mallein.	Complement fixation.	Mallein.	Complement fixation.	Post-mortem.	Post-mortem.
		+	+	–	–	–	+	+	–	?	–	?	+	+	–
Montana	51	23	23	13	13	4	4	8	8	3	3	....	....	....	....
North Dakota	37	19	19	....	....	11	11	....	....	1	1	6	6	....	....
Michigan	15	13	13	....	....	....	....	1	1	1	1	....	....	....	....
Illinois	10	4	4	....	....	....	....	2	2	....	....	4	4	....	....
Missouri	8	1	1	....	....	....	....	....	....	5	5	2	2	....	....
Connecticut	5	4	4	....	....	....	....	1	1	....	....	....	....	....	....
Florida	20	5	5	1	1	8	8	....	....	6	6	....	....	....	....
Maine	51	....	....	32	32	....	....	7	7	12	12	....	....	....	....
Washington, D. C.	93	....	....	....	....	....	....	....	....	....	[1] 76	....	17	17	....
Do	118	....	....	....	....	....	....	....	....	....	[1] 116	....	2	2	....
Miscellaneous	379	....	....	....	....	....	....	....	....	....	377	2	2	2	....

[1] Horses are normal two months after the test.

Out of the total of 787 cases shown in the foregoing table, 199 were tested by both the mallein and the complement-fixation methods, while the remaining 588 cases were diagnosed by the latter method alone. Of the 199 cases in which the two tests were compared, the mallein test was confirmed by the complement-fixation test in 115 cases and was not confirmed in 42, while the remainder gave atypical reactions to mallein but were definitely diagnosed by complement fixation.

In order to determine whether the fixation of the complement may be obtained occasionally in normal horses or in horses affected with various diseases other than glanders, a number of tests were made with the blood of apparently normal horses, and also with horses suffering with various infectious and noninfectious diseases. One of these tests was made with the blood of a horse affected with swamp fever, in which the temperature registered 106.2° F.; other tests were made with blood from horses affected with distemper, influenza, pneumonia, heaves, lameness, fistulous withers, forage poisoning, etc.; but in all these cases negative reactions were obtained.

From these results the specificity of the test can be readily appreciated. Animals affected with glanders will give a positive reaction. Normal animals or those affected with diseases other than glanders will give no reaction.

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Issued June 12, 1911.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 137.
A. D. MELVIN, CHIEF OF BUREAU.

ANTHRAX,

WITH SPECIAL REFERENCE TO THE PRODUCTION OF IMMUNITY.

BY

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UNDER THE DIRECTION OF

JOHN R. MOHLER, V. M. D.,
Chief of the Pathological Division,
Bureau of Animal Industry.

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1911.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., March 20, 1911.

Sir: I have the honor to transmit for publication the accompanying manuscript on the subject of anthrax, by Dr. Charles F. Dawson, of the Delaware College Agricultural Experiment Station, the experimental work having been carried out in cooperation with the Pathological Division of this bureau.

Anthrax is one of the oldest and most widely distributed diseases of animals, and is particularly destructive to cattle and sheep. The existing method of combating the infection is to endeavor to control it by means of a vaccine devised by Pasteur. Two injections are, however, necessary by this method, and an active immunity is not established for about four weeks.

Two important results have been accomplished through the work described in this paper. A single vaccine has been produced which may be substituted for the above-mentioned double vaccine, thus cutting down the time one-half. The other result is the production of an antianthrax serum that will confer an immediate passive immunity. Thus the two combined are a distinct advantage over the existing method in the case of sudden outbreaks, where there is no time to prepare for the disease in the usual way. This serum can also be used in conjunction with the double vaccine of Pasteur.

I recommend that the paper be published in the bulletin series of this bureau.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page.
- Introduction 5
- Historical 5
- The anthrax bacillus 6
  - Cultural and morphological characters 7
- Channels of infection 9
- Infection of the soil 10
- Methods of diagnosis 11
  - Preparation of specimens 11
  - Animal inoculations 12
- Different forms of anthrax 13
- Symptoms 14
- Post-mortem appearances 15
  - The cause of death 15
- Disposal of carcasses 19
  - The official method in Delaware 17
- Susceptibility of the various animals 17
- Anthrax in man 19
  - Malignant pustule 19
  - Pulmonary and intestinal anthrax 20
- The anthrax season 20
- Prevalence of anthrax in Delaware 22
- Methods of producing immunity in animals 24
  - The existing methods of double vaccination (Pasteur method) 24
    - Effectiveness of the method 25
    - A test of Pasteur vaccines 26
- Experiments with various substances to test immunizing powers 28
  - Anthraxin 28
  - Pyocyanase 30
  - Anthraxase 35
  - Anthraxoin 38
  - A commercial vaccine in pill form 40
- Preparation of an effective single vaccine 41
  - Tests of the single vaccine 42
- A serum for producing immediate immunity 43
  - Experiments with the serum 44
- Conclusion 47

ANTHRAX,

WITH SPECIAL REFERENCE TO THE PRODUCTION OF IMMUNITY.

INTRODUCTION.

Anthrax is primarily a disease of herbivorous animals, and occurs as an epizootic in sheep, cattle, horses, and mules, the animals being susceptible in the order named. In experimental work the small animals, such as mice, guinea pigs, and rabbits, are extremely susceptible. The French name for the disease is "charbon;" the German, "Milzbrand." When it occurs in domestic animals it is also sometimes known as splenic fever. In natural outbreaks anthrax usually has the character of a rapidly fatal septicemia, with the presence of large numbers of the characteristic bacilli in the blood.

While the disease does not occur as a natural infection in man in the same sense as in animals, man frequently becomes infected from handling infected animals or their products, such as hides and hair. When man becomes infected through abrasions of the skin, the resulting disease process is carbunculous in nature and is known as "malignant pustule." When the infection takes place in the lungs, the disease is known under the name "woolsorters' disease." A third form in man may attack the intestinal tract. The latter two forms are rapidly fatal. In malignant pustule the disease may remain localized and the patient may recover. If, however, the bacilli migrate from the local lesion to the blood stream, death rapidly ensues, the patient dying from septicemia or toxemia.

HISTORICAL.

Anthrax is one of the oldest diseases. Moses records it in Exodus 9:9. Homer, Ovid, Plutarch, Virgil, Pliny, and many others have mentioned it in their writing. It exists in all countries and in all latitudes. It was formerly very destructive to human life as well as to animals. In 1617, near Naples, 60,000 people are reported to have died from anthrax. In San Domingo, in 1770, 15,000 persons perished in the short period of six weeks. This enormous death rate was probably due, in part, to eating the carcasses of animals dead from the disease.

It was not until the middle of the nineteenth century that scientists began the serious study of this very destructive disease. In 1849, Pollender, while studying the blood of animals dead from anthrax, discovered numerous rod-shaped, microscopic bodies, which he claimed were the cause of the disease. This claim remained unsubstantiated until 1863, when Davaine announced that the bodies discovered by Pollender were bacteria, and showed that the blood of an animal could not cause anthrax in another animal when injected into it unless it contained these bodies. To this bacterium Davaine gave the present name, *Bacillus anthracis.* Davaine's views were not immediately accepted, but others took up the work, and in 1876 Dr. Robert Koch, in making his first contribution to bacteriology, announced that he had been successful in proving the correctness of Davaine's work on anthrax, and the question was then accepted as settled. Koch isolated the bacilli in pure culture and demonstrated their ability to form spores. He claimed that natural infection takes place through the intestinal tract, although he was at first unable to demonstrate this by feeding food infected by artificial cultures. Later, Pasteur succeeded in producing anthrax by feeding hay sprayed with cultures of *Bacillus anthracis* when he at the same time mixed with the hay thistles or any plants that would cause pricking or abrasions of the intestinal tract so that the bacilli could pass into the tissues.

THE ANTHRAX BACILLUS.

On account of the comparatively large size of the *Bacillus anthracis*—it being from 5 to 20 micromillimeters long and from 1 to 1.5 micromillimeters broad, although both longer and shorter forms occur—and the great readiness and certainty with which it kills experimental animals, it was the earliest disease to be studied with the microscope and by modern bacteriological methods. We first learned to demonstrate spore formation and spore staining from anthrax cultures. The germ is exceedingly easy to cultivate, growing on almost any medium and at a wide range of temperature. It has many of the general morphological characters of the whole group of bacteria, and is, therefore, very useful for teaching purposes. The bacillus has a place in the earliest researches on immunity, Pasteur having shown in 1880–1882 that animals inoculated with strains of the bacilli which had been devitalized to a certain extent by cultivation at high temperatures did not contract anthrax when exposed, while those not so protected did contract the disease. Spores of anthrax are among the most resistant things in nature, and are, therefore, useful in testing the germicidal power of disinfectants.

If a drop of blood is taken immediately after death from an animal dead from anthrax and examined with a microscope, large numbers of nonmotile bacteria will be seen. A much better view of them can be obtained if a so-called blood-smear preparation is stained with methylene blue or any of the basic anilin dyes. It will be noticed that the ends of all the bacilli are sharply cut across. If viewed with a higher power, it will be seen that the ends bulge at the corners, making the ends appear slightly concave. The cells are also noticed to have a capsule, and when several cells are lying end to end the capsule seems common to the entire chain of cells.

If in making preparations of anthrax blood great care is taken not to overheat, but just enough to "set" the specimen, and methylene blue is used as the stain, we can, while viewing the slide by reflected light with the unaided eye, notice a violet coloration of the entire specimen. If we now place the slide under the microscope, we can see that the space between the bacillus and its capsule is stained a reddish color. This is known as McFadyeans color test, and if the specimen is properly prepared is to be observed in all anthrax preparations made from dead animals. The test is most successful in those preparations where the bacilli are very numerous, and could be of great importance in field work where a microscope may not be at hand.

CULTURAL AND MORPHOLOGICAL CHARACTERS.

The bacillus of anthrax has such definite and constant cultural and morphological characters that it can hardly be mistaken for any other bacterium. When growing on the surface of agar-agar plate cultures beautiful colonies occur, appearing as wavy wreaths resembling locks of hair or bits of raw cotton thrown on the surface. It is supposed that the whole mass consists of thousands of contiguous cells forming one long chain, wound up in all directions.

The growth in gelatin tubes is characteristic in that it liquefies the medium and the growth resembles an inverted fir tree.

In milk a small amount of acid is formed, so that milk is slightly coagulated and slowly peptonized.

In peptone bouillon virulent bacilli make a much more luxuriant growth than do the attenuated bacilli or vaccines. The heaviest growth is near the surface, where it can obtain oxygen, and consists of a dense mass of intertwined bacilli in chains which readily fall to the bottom of the tube if it be even slightly shaken. This heavy growth does not occur when bouillon is inoculated with attenuated bacilli.

Growth on all media proceeds best at 35° C. The minimal temperature for growth is 12° C., and the maximal 45° C. The vegetating bacillus is killed by a short exposure (15 minutes) to 60° C. moist heat, and by desiccation in a few days at ordinary temperatures. The spores, on the other hand, will stand almost any disinfecting process that any infected material can stand without greatly changing its physical characters. Hence it is impossible to disinfect hides containing anthrax spores without spoiling them for leather making. Spores require a boiling temperature in moist heat, and a much higher temperature in dry heat to destroy them. Spores live for a long time—probably 10 years—in the soil or in water, and it is because of this great resistance that pastures once infected remain so for an indefinite period.

The bacillus can grow either in the presence or absence of oxygen, but it grows best when air or oxygen is present. Spores can form only in the presence of oxygen, and this accounts for the fact that the bacillus never sporulates in the living blood nor in cultures grown under anaerobic conditions.

When spores begin to form, a tiny bright spot appears in the center of the parent cell, which soon increases to the diameter of the cell and always remains in its center. The cell protoplasm seems to be used up in the production of the spore, which now forms the resting stage of the organism. This spore remains quiescent until it is again placed in a suitable environment for growth, when it sprouts and assumes the bacillary or infective stage.

Although the spore, or resting stage, of *Bacillus anthracis* is very resistant to all natural and artificial disinfectants, the bacillar or infective stage is quite vulnerable. The bacilli locked up in the airless blood vessels or cavities of the body not only do not spore, but rapidly disintegrate after death. These important facts are of incalculable value in the suppression of the disease by nature, and furnish the strongest kind of an argument for the prompt burial of animals dead from the disease. If such an animal be opened, however, the above-mentioned natural protection against the spread of the infection is removed, as the bacilli will continue to multiply and sporulation will finally ensue.

The following experiments were made to demonstrate the foregoing facts: The spleen of a guinea pig just dead from anthrax was minced and placed in a Petri dish exposed to air at room temperature. Microscopic examinations of this material 16 hours after showed that every bacillus had spored, the protoplasmic cellular material staining poorly. Another experiment made to determine the effect of such exclusion of the air as burial can afford showed that 24 hours are required for spore formation.

In order to determine the effect of total exclusion of air upon the question of viability of the bacilli, capillary glass tubes were filled with heart's blood from a mouse just dead from anthrax and sealed in a flame. These sterile tubes were filled under precautions which prevented the ingress of extraneous bacteria; hence there were only anthrax bacteria in them, and the disintegrating effects of post-mortem bacilli through the septic ferments which they form in a decomposing carcass were not present. Examinations and cultures from these tubes were made at intervals up to the twenty-third day, and while the bacilli were lessened in numbers, they were still viable. In buried carcasses, even though they be not opened, putrefactive bacteria grow very rapidly and their excretions cause a granular degeneration of the anthrax bacilli. If air is admitted to the carcass these excretions have no deleterious effect upon the spores that have formed, and such a carcass infects the soil in which it is buried, while if the carcass had not been opened, and care had been taken to disinfect the soiled exterior and air-containing cavities of the animal, the putrefactive bacteria would kill off the anthrax bacilli. The length of time necessary for this destruction varies with the temperature, being about a week in hot weather.

CHANNELS OF INFECTION.

There are four routes by which animals may become infected, namely, by ingestion, by inoculation, by inhalation, and by transmission.

Ingestion.—The first and most frequent, and therefore the most important, is by ingestion. This form is acquired when animals are turned out to pasture.

Inoculation.—The method of infection next in importance is by inoculation, which is brought about by contact of abrasions and wounds with infected material, or by flies or other insects.

A case of spontaneous inoculation arising in the course of my experiments—the only one which occurred in the work—was where a ram, immune to anthrax, was severely bled to furnish blood for experiments. He was kept in a stall where a sheep had died three months previously of experimental anthrax. A considerable wound was made by the operation and the animal was found dead of anthrax on the second day after having been bled.

Inhalation.—The form third in importance is by inhalation. Here spores are breathed in with dust arising from infected material or surfaces.

Transmission.—A fourth form, which is probably rare, is that of transmission from the mother to the fetus in utero. I have observed this in the case of an experimental cow which had been immunized with a single anthrax vaccine and subsequently tested with virulent bacilli. The mother at no time appeared sick, but on the sixteenth day after receiving the virulent bacilli she dropped a dead calf, which appeared to be a full-term one. Not suspecting the cause of the trouble, the calf and membranes were buried. Fortunately, a loop of the discharge found on the ground was plated and developed a pure cultivation of 60,000 anthrax bacilli, the virulence of which was demonstrated on a guinea pig. The cow was in no way sick, and daily microscopic examinations of the vaginal discharges showed these were free of anthrax bacilli.

INFECTION OF THE SOIL.

Soil becomes infected with anthrax by discharges from animals suffering with the disease, and by the dead bodies of animals which are allowed to decompose upon the surface. These discharges consist of small hemorrhages which occur from the mucous membranes in the nose and bowels, mixed with the nasal secretions and with the feces. These sometimes do not occur until just before death. In some cases the discharges are so limited as almost to escape observation, but a microscopic examination in such cases would reveal large numbers of bacilli. Hence it is very important to limit to the smallest area possible the wanderings of an animal so affected. It is doubtful if the feces of the smaller experimental animals contain anthrax bacilli. One observer reports the presence of anthrax spores in the feces of guinea pigs, but I have never found them.

Pasteur, in his memorable paper on anthrax before the Academy of Sciences in Paris in 1880, gave his opinion as to another manner in which the surface becomes infected with this disease. He said in part:

> Earthworms are the bearers of the germs, and they bring this terrible parasite to the surface of the ground from the depths to which it has been buried. It is in the little cylinders of earth, of fine earthy particles, that the worms excrete and deposit at the surface of the ground after morning dews, or after rains, that are to be found, besides a crowd of other germs, the germs of charbon (anthrax).

While it does not seem impossible that earthworms may swallow the spores of anthrax and cast them out upon the surface, that close observer, Koch, was unable to verify the facts. He held that pastures remain infected on the surface from preexisting cases, and that the parasite remains on or near the surface from season to season and infects animals which graze upon such soil. When the grass gets short in dry weather, spores that may have been carried into the soil by previous rains either rise again by capillarity or are brought to the surface by the animals pulling the grass up by the roots. The writer has examined many earthworm casts from anthrax graves of various ages—from 15 years to 1 month—and has failed to find anthrax spores in them. Pasteur's work was done before the days of solid media, and hence the plate-culture method of isolation was not used by him. Another disease, malignant edema, is easily produced in guinea pigs and rabbits, and greatly resembles anthrax clinically, as well as in the morphology of the organism causing it. These two germs would greatly resemble each other when viewed with a microscope made in 1880.

In order to test further the question as to whether the surface above an anthrax grave becomes infected by earthworms, or otherwise, the writer has for the past three years pastured sheep and cattle upon soil in which there are many anthrax graves, and no case of spontaneous anthrax has arisen. The animals were frequently observed grazing in the little hollows formed by the sinking in of the graves, and it would seem that this is sufficient proof of the questionableness of Pasteur's early theory regarding this source of infection.

METHODS OF DIAGNOSIS.

Anthrax is a disease that we absolutely know is caused by the parasite ascribed to it. We can tell just how long an animal will live after it has been inoculated with a given culture, provided the culture is of maximum strength. We know that a virulent culture will, in proper dose kill the laboratory animals, as well as sheep, in 48 hours, and that we can always recover the germ in the blood of such animals and reproduce the same disease in another animal by inoculating it with this blood. Hence, all of Koch's postulates can be fulfilled.

Different races of anthrax bacilli vary in virulence. Those of highest virulence will kill guinea pigs and rabbits in 0.00001 c. c. doses of a 24-hour bouillon culture in 48 hours, and the same culture would kill a sheep in 95 hours when given a 0.0001 c. c. dose subcutaneously. Very young animals are much more susceptible to anthrax than adults, and hence care must be exercised in vaccinating young animals. A sucking rabbit a few days old died in 18 hours from a 0.1 c. c. dose of bouillon culture, and the virulence of the bacillus, which had become somewhat lessened, was greatly enhanced.

A diagnosis can be made by the veterinary scientist by two methods; either by a bacterioscopic examination of blood smears made soon after death or by animal inoculations. On account of the nature of the disease it is highly desirable that the first method be used, as no time should be lost in coming to a decision. It is easy to reach a decision if the specimen has been properly prepared and if the microscopist is familiar with the organism.

PREPARATION OF SPECIMENS.

A small drop of blood thinly spread on a piece of glass or paper and dried immediately is all that is required. It is best to obtain blood for this purpose by making a needle puncture into the jugular vein, as sometimes the bacilli are not numerous in the peripheral blood vessels. A specimen prepared in this way will arrive at the laboratory just in the condition in which it was collected. There will be no bacteria in it that were not there when it was prepared.

Sometimes specimens arrive consisting of two smeared surfaces of glass stuck together. These are dangerous, and it is next to impossible to separate them. They should be boiled and thrown away, no attempt being made to make an examination.

The most dangerous anthrax package, and one which is not allowed in the mails, is the bottle of blood. These have arrived broken, and in one instance a positive diagnosis was made from blood smeared on the outside of the package by the sender's hands. Again, when parts of organs, or blood in volume, are sent in, decomposition will have taken place and a diagnosis may be impossible. The fruit-jar package, containing spleen, liver, heart, etc., sent by express, is another most dangerous package. These have arrived broken, with liquid dripping from them. When not broken their opening is generally attended with dangerous difficulties. The top is usually screwed down so tight that considerable force must be used to open it, and when this is accomplished the gases which have formed will spray the liquids upon one's hands and clothing.

A diagnosis of a blood-smear preparation is made by first "setting" the specimen by heat, passing the glass slide through the flame three times, occupying a second each time. The specimen is now stained with methylene blue or fuchsin, dried between two pieces of filter paper, and mounted for examination. If anthrax bacilli are present they can be easily seen stained blue or red, according to the stain used. They appear as rods with square ends. They are never round or blunt. They will almost always appear in chains, although they frequently appear as single cells when torn apart in making the preparation. Spores are never found in a specimen prepared from a recently dead animal. With lenses of 1,000–1,200 amplification the ends appear somewhat concave, from bulging of the corners of the cylinder. When such an organism is found in the blood of an animal recently dead the disease may be called anthrax, as it is the only one in animals that provides such a picture.

ANIMAL INOCULATIONS.

When there is reason for doubt, animal inoculation must be resorted to. Mice, guinea pigs, and rabbits may be used, and these are susceptible in the order named. The suspected material is ground up in a sterile mortar containing normal salt solution. The solid particles are allowed to settle or are filtered off, and a few drops to 0.5 c. c. of the liquid are injected subcutaneously into guinea pigs. If anthrax be present the animal will die in two or three days, and the bacillus can be recovered from its blood and examined as in the first method. Plate cultures should also be made from the extract of the suspected substance, as it is possible to isolate the anthrax organism and thus come to a decision from a study of the anthrax colonies if any develop, as such colonies have a characteristic appearance. Inoculation into experimental animals of cultures made from these colonies, if they be anthrax, will cause the disease, and thus any doubt as to their identity will be removed.

DIFFERENT FORMS OF ANTHRAX.

Anthrax manifests itself in different forms according to the seat of invasion, kind of animal, virulence of the bacillus, season of the year, and time of the outbreak.

The most acute type of the disease is seen oftenest in cattle and sheep and is known as apoplectic or fulminant anthrax. This form appears suddenly, without premonitory symptoms. The animal is suddenly seized with trembling, a haggard expression, swaying, difficult breathing, and cyanosis. Convulsions and death occur in from a few minutes to two or three hours. Bloody discharges usually occur during the last hour from the nose and rectum. These are very infectious, as the bacilli are present in large numbers, and constitute an important way in which pastures become infected. For this reason such animals should be confined where these discharges can be disinfected.

The second form, known as anthrax fever or internal anthrax, differs from the fulminant or apoplectic form only in its duration. The symptoms in the last stages are practically those seen in the most acute type, except that they are more intense and of longer duration. There will be excitability, restlessness, high fever, oozing of blood from the nose, eyes, ears, rectum, and thinner parts of the skin of the axilla or thigh; tremors, dullness, prostration, grinding of the teeth, colicky pains, difficult breathing, arching of the back, rolling of the eyes, convulsions, and death in sheep in 24 hours, in cattle in from 2 to 5 days, and in horses in from 1 to 5 days.

The third form is local or external anthrax. In this form the disease is at first localized in the mouth, throat, or skin in cattle, and the same form appears most often in horses in the tongue, throat, neck, breast, withers, shoulders, flank, or thigh. These swellings are firm or doughy, insensitive at certain parts, and tend to become gangrenous. There is no tendency to suppuration, and they do not crackle on pressure—a diagnostic point when there is doubt as to whether the case is one of anthrax or of blackleg. When cut into—something which should never be done, as the exudation always contains the anthrax bacillus—there escapes either a pale, straw-colored, or bloody liquid.

SYMPTOMS.

The symptoms of anthrax will vary with the species and with the type of the disease, except that in the last stages of any of the three types the most pronounced symptoms are identical. In the most acute type the animal may appear at first to be perfectly well and keep along with its fellows even when its temperature is very high—as high as 106° F. Along with such a temperature we shall of course find a rapid pulse and increased respiration. When one is standing close beside such an animal the heart beats may be plainly heard. Soon other symptoms, such as grinding of the teeth, tremors, and standing with head down, appear. Then appear drooping of the head and ears and a disposition to lie down. Animals that have been lively will now decline to rise unless handled roughly. They become stupid and sleepy and very weak in the hind parts. Whereas the temperature has all along been high, it now shows a sharp decline, and before death may become subnormal. The visible mucous membranes are a dusky red, especially those of the rectum and vulva. There is a bloody nasal discharge. The feces will be coated with a bloody mucus. Local swellings appear in the mouth, throat, neck, and breast (especially in horses), and there are sharp attacks of colic and convulsions which end the misery of the animal in from 12 to 48 hours after the disease is first noticed. Pregnant animals are liable to abort and thus greatly spread the infection through the copious discharges. An outbreak has its highest mortality at its onset, while later on some animals, especially horses and mules, may recover. In the most acute types, which occur mostly in cattle and sheep, the animal is found dead. A cow which seemed well at night is found dead in the morning, or if death occurs in the daytime, the illness is of short duration, occupying only a few minutes or one or two hours. In these sudden attacks the symptoms follow each other so rapidly and death is so sudden that sometimes the owner is convinced that the animal has been poisoned. The attack is ushered in with trembling, anxious expression, high fever, rolling of the eyes, and convulsive movements, soon followed by general convulsions and death.

In the local form or cutaneous anthrax in cattle, swellings appear suddenly on different parts of the body at one or many places, and the animal dies with the same symptoms as occur in the most acute type when the bacilli reach the circulating blood from these local lesions. When the infection occurs in the tongue or pharnyx, we have in the first case gloss anthrax, and in the latter pharyngeal anthrax, the symptoms varying somewhat according to the part most affected; but the general constitutional symptoms will be those already described. In some cases the most prominent symptoms at first will be enormous swellings of the rectal mucous membrane.

In the local form, or cutaneous anthrax, in the horse, the swellings occur at the points of entrance of the bacilli or spores, where there are abrasions of the skin or mucous membrane, or where biting insects have brought the infection from a previous case. These swellings appear suddenly at the point of inoculation and are characterized by a rapidly spreading edema. The general symptoms are not so urgent, the fever is less intense, and the mortality, while not so great as in the more acute form, is still high.

POST-MORTEM APPEARANCES.

An animal that has died of anthrax will nearly always be found much bloated, with blood oozing from the nose and rectum. There will be evidences on the ground that the animal died a violent death, in convulsions. Local swellings will be present or absent according to the type of the disease. In the rapidly fatal cases little change will be noted either in the blood or internal organs beyond those produced by high blood pressure, indicated by a swollen spleen and engorged liver. The carcass itself—the edible portions—would show nothing that would make it doubtful as food. In the more prolonged cases a hemorrhagic condition will be noted in all the internal organs. The blood will be tarry in appearance and will not clot. The heart is often light in color, while on the inside it will be found deeply stained and containing dark, uncoagulated blood. The liver may be found enlarged and is easily torn in handling, presenting on its surface hemorrhagic areas. The spleen is often specially enlarged and distorted in shape, and ruptures on handling.

The bacilli of anthrax can be found in largest numbers in those organs where the lesions are most pronounced, namely, the spleen, liver, and engorged lymphatic glands, but they are commonly found in any part of the vascular system. In the serous cavities, such as the pericardial, the pleural, and the peritoneal, may be found a sanguineous fluid consisting of serum, red and white cells, and anthrax bacilli. The hyperemia and areas of extravasated blood may appear at any point of the body where the bacilli have become localized and enormously multiplied, forming capillary embolisms, which consist of broken-down blood corpuscles, and bacilli. Hence they are frequent in the tongue, throat, lungs, stomach, and intestinal walls; the mesentery and the omentum; the skin, connective tissue, and the muscles.

THE CAUSE OF DEATH.

From the manner of death it would seem that the poison of anthrax acts specifically upon the center of respiration, this in turn allowing a fatal accumulation of carbon dioxid in the blood. That it is the replacement of the oxygen of the blood by carbon dioxid that is the immediate cause of death in anthrax is further shown by the loss in the blood of the property of coagulation, it being known that carbon dioxid precipitates and throws out of action the fibrin-forming element of the blood—fibrinogen.

DISPOSAL OF CARCASSES.

The disposal of an anthrax carcass should not be left to the owner nor should anyone be allowed to open a carcass of an animal that is supposed to have died from anthrax. The diagnosis can only with certainty be made by a competent bacteriologist. He need only be furnished a drop of blood from the suspected animal. This blood can be drawn from the jugular by means of a syringe, or a small nick sufficient to allow a drop to escape may be made with a suitable instrument. This blood should be smeared upon a piece of glass or piece of paper, dried immediately, and forwarded to the bacteriologist. Any blood escaping during the above operation should at once be disinfected. The State should appoint a sanitary agent in every election district, whose duty it should be to dispose of anthrax carcasses, according to instructions issued by a competent authority, as it is only by the proper disposal of the carcass and by vaccination that the disease can be controlled. While the majority of our farmers recognize the advisability of promptly burying the carcass, there are those who would allow the animals to lie where they die to decompose or be eaten by dogs and buzzards. Parts of the carcass may be carried by dogs to adjoining or distant pastures and spread the infection. Spores forming in the carcass opened in this way would pass through the intestinal canal of buzzards and dogs and permanently infect pastures miles away. The soil upon which such an animal has lain would certainly be infected and remain so for many years.

During the last few years deep burial, rather than efforts at cremation, has been practiced, with good results so far as can be ascertained. While cremation properly carried out is undoubtedly the safest method of disposing of an anthrax carcass, it is practically impossible to destroy absolutely all vestiges of the body and bacilli-laden body fluids, and unless this is done the whole operation so far as it goes is almost a useless waste of time, material, and labor. In the cremation of carcasses in the field even the earth upon which it has lain should be thoroughly and deeply burned over so that the heat will penetrate to a depth sufficient to insure the death of the anthrax bacilli which certainly passed into the soil with the body fluids when rupture occurred from the heat. We know enough of the biology of the anthrax bacillus to take advantage of the fact that if it is kept locked up in the closed cavities and blood vessels of the body where no air can reach it, not only will the bacilli fail to produce the resistant spore, but they will actually decompose and become noninfectious, so that the bacilli which are in the blood vessels of an unopened animal perish. On the other hand, those bacilli which have come away in the pulmonary and rectal discharges during life will, if they are provided with sufficient material to keep them moist, promptly go into the spore or resting stage. These constitute the main source of danger, and in this way a considerable area may become infected. For similar reasons, hemorrhages into air-containing cavities, such as the lungs, trachea, head, sheath, vagina, and rectum, all favor the production of the resistant spore. Hence, if we introduce into the cavities a disinfectant which will surely kill the spores, and also at the same time take care to disinfect the soiled exterior surfaces of the animal, and then bury such an animal without opening it, we may reasonably conclude that such a carcass will not thereafter be a source of infection.

THE OFFICIAL METHOD IN DELAWARE.

In Delaware the method of procedure as advised and as carried out by the State board of agriculture is as follows: Formalin is injected in both directions into the trachea. Formalin-soaked cotton is pressed up the nostrils and into the mouth, rectum, vagina, or sheath. The grave is dug where the animal dies, when possible. The body of the animal is then either thoroughly wetted down with a 4 per cent solution of formalin or kerosene, or coal oil is poured over the uppermost side of the animal and is set on fire. Only enough kerosene is applied to burn off the hair, as it would defeat the purpose to burn the animal sufficiently to cause rupture of the body. The carcass is then rolled into the grave with the other side uppermost. This side is now sprinkled with the oil and fired, after which the dirt upon which the animal has lain is disinfected with formalin and is then shoveled into the grave first. The grave is now filled and banked up as is the custom. Such graves should be surrounded by a fence. In fact, there should be a graveyard widely fenced in, and when an animal is known to have anthrax, it should be placed therein and allowed to die where it is to be buried. Such an inclosure should never be used for any other purpose, and should be so located that drainage from it upon other land will not occur.

SUSCEPTIBILITY OF THE VARIOUS ANIMALS.

While all the farm animals and even man himself will contract anthrax, we know that the degree of susceptibility varies not only in the different species, but also in individuals of the same species. We find that the herbivora are very susceptible and that the carnivora are less so, while the omnivora occupy a place between these two extremes. Cattle and sheep, being extremely susceptible, contract the fulminating type in nearly every case, while horses and mules frequently live several days, exhibiting the local or skin form, and some cases recover. It could probably be shown that any ruminant is more susceptible than other herbivora because of the anatomical and physiological characteristics of their respective digestive organs. In ruminants we find the immense rumen, with its moisture, heat, and alkaline reaction, an admirable incubator for bacteria. The outward passage of the contents of the rumen is slow, so there is plenty of time for anthrax spores swallowed with the food to develop into bacilli and multiply into prodigious numbers before they pass through the other two stomachs into the true or acid stomach, where some of them may be digested, but where many may pass out unharmed into the alkaline intestine and be absorbed with the food, finally entering the blood stream suspended in the chyle. It would now require only a few hours for them to multiply in the blood into sufficient numbers to produce their fatal poison. It is doubtless in this way that cattle which seem well when last seen and fed at night come down suddenly with the disease and are found dead in the morning.

Swine are less susceptible than horses and cattle, yet when swine eat the carcasses of animals dead from anthrax they may contract the disease. As in other species, the young sucking pigs are more susceptible than the adults. As swine usually contract the disease by eating the dead animals, they generally have the disease in the throat or intestine. The symptoms in such cases do not vary materially from like cases in the other species.

Dogs and cats contract the disease similiarly to hogs and are classed among the less susceptible. The symptoms are those to be expected, and consist of swelling of the throat, difficult swallowing, vomiting, profuse bloody diarrhea, high fever, and death.

Birds of prey are said to be immune to the disease. Chickens are less susceptible than swine, yet Pasteur showed that chickens were quite susceptible when immersed in cold water and then inoculated. Likewise frogs, which are ordinarily immune, can be successfully inoculated after immersion in warm water. Young pigeons of certain breeds are easily infected artificially, and sparrows, finches, canaries, yellow-hammers, and redbreasts have been successfully inoculated.

In chickens anthrax runs a very rapid and fatal course in 24 hours. They can contract the disease by eating the carcass or discharge of an animal dead from anthrax. As in other animals, a differential diagnosis between this disease and other chicken diseases of like type should be based upon the discovery of the anthrax bacillus in the blood along with the symptoms of sudden debility and high fever. Anthrax swellings will occur on the comb and wattles, around the eyes, and on the tongue, palate, and feet. The bird is extremely weak, has violent tremors and convulsions, and bloody diarrhea, and dies within 24 hours.

ANTHRAX IN MAN.

Anthrax is not, strictly speaking, a human disease, yet it is not rare in the practice of physicians who attend the workers in tanneries where imported hides are converted into leather, nor is it rare in persons whose occupation brings them in otherwise contact with hides, wool, hair, furs, hoofs, bones, rags, felt, glue, or any product made from the bodies of animals that die of anthrax. Hence the disease is found in shepherds, cattlemen, horsemen, farmers, drovers, butchers, tanners, brush makers, and veterinarians.

The disease is notoriously common in those who handle imported hides, although man can become infected through the bites of flies or other infected insects. The sound skin is sufficient protection against the entrance of the bacillus, but the slightest abrasion is sufficient as a point of inoculation in handling infected material, or even for inoculation by the ordinary housefly that has visited the discharges of an animal suffering with anthrax. The process of tanning does not always disinfect an anthrax hide. That hair is a medium of infection is shown by outbreaks of anthrax among brush makers. Persons who handle imported wool and rags contract the disease by inhaling the spores in the dust which arises. This spore develops into the bacillus, and a case of pulmonary anthrax is established. This form is known as woolsorters' disease, or ragpickers' disease. Those who handle bones and carcasses in fertilizer, glue, and rendering establishments located in anthrax districts are peculiarly exposed to infection. In man the large majority of cases occur on the face, probably because this part of the body is more liable to be attacked by biting insects.

Anthrax in man is a local disease, and we do not find the bacilli in as large numbers in the blood or other organs, such as the liver, spleen, and kidneys, as we do in the lower animals. The bacilli can, however, be found in abundance in the local lesion.

MALIGNANT PUSTULE.

Malignant pustule, or local anthrax of the skin, usually occurs on the face, hands, arms, or neck of those who handle imported hides. In from one to three days after infection takes place a small red pimple appears. This soon changes to a vesicle, which is very painful. The center of the vesicle rapidly becomes necrotic, forming a black eschar, which soon becomes surrounded by a ring of vesicles.

The surrounding tissues become congested and edematous, and the lymphatics are involved. The fever is quite high, and the patient is very sick from the absorption of poison from the local disease. It runs a rapid course, and the patient may die from toxemia.

Most of these cases are, fortunately, susceptible of successful treatment. This consists of early and thorough excision of the pustule and all infected surrounding tissue, followed by the local application of strong disinfectants. At Guy's Hospital, in London, 13 out of 15 cases were cured by excision, even though in 12 of these cases the inflammation had spread to the surrounding parts or had involved the lymphatic glands with more or less severe constitutional disturbance. In some cases the disease process goes no further than the formation of the eschar, which then becomes a scab, with subsidence of the inflammatory process, and recovery. In the majority of cases, however, the disease runs a regular course; if not excised early the bacilli enter the blood stream and the case terminates fatally from a modified form of the same disease as occurs in cattle, with the exception that the bacilli are not found in large numbers in the internal organs nor is the spleen greatly enlarged. It may, therefore, be said that in man death is largely due to the absorption of toxins from the local lesions. When the pustule is situated on the extremities the percentage of recoveries under treatment is large.

PULMONARY AND INTESTINAL ANTHRAX.

Pulmonary anthrax, or woolsorters' disease, is caused by the inhalation of spores by those who handle hides or other infected material. The initial lesion is local and is situated in the lower trachea. It consists of a swollen and hemorrhagic condition of the mucous membrane, with great enlargement of the mediastinal and bronchial glands and of effusions into the pleural and pericardial cavities and the lungs. Externally may occur also cutaneous edema over the chest and neck and inflamed glands. This form is rapidly fatal, the patient dying from suffocation and toxemia.

Intestinal anthrax is also of local origin and has about the same pathology and terminations as the pulmonary form. There is intense inflammation of the intestinal mucous membrane and involvement of the neighboring lymphatic glands. Intestinal infection takes place when anthrax-infected products are eaten.

THE ANTHRAX SEASON.

Anthrax is peculiarly a disease which may be said to be a seasonal one; that is, the disease makes its appearance with the advent of certain kinds of weather. In March, 1907, in Delaware we were visited with some exceptionally warm, springlike weather of considerable duration, and on the 22d of that month the first case occurred for that year, an exceptionally early start. In 1908 the first case occurred on May 2. In 1909 the first case did not occur until July 23, but the disease persisted until November 6, whereas in 1907 the last case was on September 24, and in 1908 on August 24. So that we may say the season lasts, in Delaware, from March to November, being most prevalent in the summer months. As the disease is, generally speaking, a warm-weather one, and as we know that the causative agent is present throughout the year, there must be some conditions which come with warm weather which predispose to the onset of the disease, either by affecting the receptivity of the animals or by increasing the infective properties of the germ of the disease. Possibly both conditions prevail. There can be little doubt that the resting stage of the *Bacillus anthracis*—the spore—will quickly develop into the bacillar or infecting stage under the influence of heat, moisture, and organic matter. These conditions prevail on the pasture in warm weather, and the writer has demonstrated that the germ of anthrax will vegetate in a 2 per cent hay infusion. Hence, when spores of anthrax come in contact with hay infusions in pastures, especially in meadows, there is produced a virulent culture which will infect the animal eating them.

The animals themselves are probably more susceptible to anthrax when turned out upon fresh pastures, as the ingestion of rank grasses, which are acid in reaction, lessens the normal alkalinity of the blood and thereby increases the susceptibility to anthrax. This greater alkalinity of the blood in carnivora may be the cause of their greater resistance to the disease, while the loss of alkalinity of the blood by the herbivora, and especially by ruminants such as cattle and sheep, through the ingestion of such large quantities of acid grass, may account for their increased susceptibility during the grass-growing season. At all events there are certain weather factors which determine, in a measure, the onset of the anthrax season, and given such conditions the season can be predicted with tolerable certainty. This season exists when we have had a long, dry period, followed by light rains or infrequent heavy rains, and then by extreme heat. This kind of weather will not only produce bacterial multiplication, but will cause a rapid growth of rank acid grasses, and these when eaten will increase the susceptibility of animals to anthrax by decreasing the alkalinity of their blood.

Anthrax, then, may be said to be a pasture disease, which exists mostly in warm weather. If it were a stable disease, why should we not have cases occurring in winter, and why is it that the disease rarely occurs in animals that are not turned out on pasture? We rarely see a case of anthrax in a carriage horse that is fed in the stall, but it is not of infrequent occurrence in horses that are turned out to pasture. Cases are rare in city horses, but common in the farm horse. The city horse must eat the same hay and grain, generally speaking, as the farm horse. If it were the hay and grain that produce the disease, we should find anthrax as common in city stalls as on the farm.

Anthrax rarely occurs in small towns. It is distinctly a disease of animals that graze on infected fields. A recent case emphasizes this. An expressman delivered some goods to a farmer who lives 1 mile from this town (Newark, Del.). This farm has anthrax every season. The expressman's horse must have grazed upon infected soil while there, as it was sick within 48 hours with high fever and edematous swellings at the root of the neck and down the front legs. A microscopic examination of the exudate showed the presence of anthrax bacilli, and the animal died in a few hours. This animal lived in the stable with others, and was fed the same feed. No case has occurred since, and it was the only animal from that stable that visited the infected farm. The great importance of an early diagnosis and isolation was demonstrated in this case. Within a few hours after a swelling was noticed anthrax was suspected and some of the bloody subcutaneous exudate was aspirated and brought to the writer by the attending veterinarian for diagnosis. It was thus possible to remove the animal from the stable to a yard where other animals could not become infected long before any infecting discharge had taken place. The animal was embalmed, washed down with formalin solution, as previously described in this article, and buried in a six-foot grave as soon as the same could be dug. The stables have been used uninterruptedly ever since (four months), and no fresh case of anthrax has occurred.

PREVALENCE OF ANTHRAX IN DELAWARE.

Anthrax has been officially recognized as existing in this State (Delaware) since August, 1892. It is highly probable that the disease had its origin in imported hides used in the tanneries at Wilmington for making leather. Large quantities of scraps accumulate during the process of leather making, and these were sold to farmers who composted them and later spread the infected material upon the land. Since the spores of anthrax would live and retain their virulence in a compost heap, it is easy to see how the lands first became infected. While it is generally conceded that meadow pastures are most productive of the disease, the tillable lands were probably infected first, as above stated. The meadow pastures have retained their infective properties because of their constant use for grazing, and particularly because of fresh cases of anthrax occurring upon them. The higher tillable lands have, however, in many cases become disinfected by the lapse of time, cultivation, and drainage.

Estimates made by veterinarians practicing in the State show that there are from 175 to 200 farms in Delaware that are permanently infected with anthrax. These farms are located, in a general way, in that territory which drains into the Delaware River and the Delaware Bay. The infected territory comprises about one-third of the total area of the State.

While anthrax has been known to exist in the State only since 1892, it is highly probable that the first cases occurred much earlier and that the disease is as old as the morocco-leather industry. The originally infected territory must have been the cultivated lands upon which infected compost fertilizer was spread by the farmer himself. Animals dying on such farms before the nature of the disease was known here were either hauled out dead or turned out while yet alive upon the marshes to die. Doubtless they would in some cases be set adrift in the creeks and thus infect any shore or marsh upon which they drifted. As the marshes are always wet and contain decaying vegetation in abundance, the conditions for the development of the microbe of anthrax are perfect, while upon the dry cultivated lands, even though the microbe be present, the conditions are, owing to the comparative absence of water, much more unfavorable to the multiplication of the causative agent. It is because of these conditions that marsh pastures are regarded as being more productive of the disease. The wash water from the tanneries at Wilmington can no doubt infect the streams into which it flows and cause the disease in animals either grazing upon them or drinking from them.

Those farms and marshes now infected will remain so for many years, even though no new cases occur upon them. This liability to outbreaks of anthrax on a farm has a depressing effect, not only upon the value of the land, but upon all agricultural operations.

While there has been no epidemic of anthrax in Delaware for several years, and the number of deaths from the disease has been exceeded by many ordinary diseases of which little notice is taken, anthrax must always be considered a menace to the agricultural welfare of the State because of the ever-present liability of an outbreak which might assume the proportion of a genuine epidemic. This liability should be offset by the annual vaccination of every farm animal in the infected territory. Even though several years may have elapsed since the last case occurred on a farm, that farm is liable at any time to be again visited by the disease.

METHODS OF PRODUCING IMMUNITY IN ANIMALS.

THE EXISTING METHOD OF DOUBLE VACCINATION (PASTEUR METHOD).

Since 1892 anthrax has been controlled by vaccination by a method devised by Louis Pasteur. This method consists in the subcutaneous injection of attenuated cultures of the bacillus of anthrax. Two injections of varying degrees of strength are made at an interval of 12 to 14 days. The first injection consists of 1 cubic centimeter of a culture that has been incubated at 42° to 43° C. for a sufficient time to decrease its virulence to a point where it will kill white mice, but not guinea pigs or rabbits. This generally requires a period of 24 days, assuming that the culture was made originally from a moist, virulent race of anthrax bacilli. Such cultures are to be made directly from the heart's blood of an animal that has died of anthrax within 48 hours after inoculation. In such blood we find only non-spore-bearing bacilli, and when these are grown at 42° to 43° C. they do not at any time produce spores while this temperature is maintained. Contrary to the statements of some, however, the bacilli promptly form spores when this temperature is reduced. These spores do not acquire any more virulence, however, than the parent bacilli which produced them.

The second injection consists of a culture similarly made and incubated at 42° to 43° C. for a period of 12 to 18 days, or one whose virulence has been reduced to a point where it will not kill rabbits but will kill white mice and guinea pigs, the latter in 3 or 4 days. The author's experience has shown that there is no hard and fast line in the number of days that a culture of a given race of anthrax bacilli must be attenuated. Much depends upon the resistance of the bacillus, the character of the culture medium, the exactness of the temperature of the incubator, and the natural resistance of the animals used in testing the vaccines. When a culture of proper strength has been obtained and is properly transferred to fresh media about once a month and kept in a cool place where it will not evaporate, it may be used indefinitely as a stock culture for inoculating a liquid medium, which constitutes the vaccine. When such attenuated cultures are inoculated into animals, a very mild and clinically unnoticeable attack of anthrax is produced, which confers an active immunity which persists throughout the anthrax season. The inoculation must be repeated the following season.

Certain precautions are necessary in the application of anthrax vaccine. Assuming that the vaccine has been properly prepared, it is the duty of the veterinarian to ascertain that anthrax is not already existing in the animals he is about to vaccinate. As a precaution, when there is reason to suspect that anthrax may already be existing, no animal showing a fever should be vaccinated, as the disease may be carried from the already infected animal to others upon the point of the inoculating needle. Again, in order to prevent abscess in horses and mules by introducing under the skin streptococci or staphylococci by the point of the needle, the place of injection should be disinfected of these microbes. The anthrax bacillus can not produce pus, but the germs usually found upon the skin are to be held responsible when abscess occurs from vaccination. The writer has been in the habit of dipping the point of the needle into strong carbolic acid contained in a small vial which may be conveniently carried in the side pocket at the time of vaccinating. None of this acid can enter the needle and kill the vaccine germs, as it is already filled with the vaccine, and none can be introduced under the skin, as it is all removed from the needle in its passage through the skin, and this in turn disinfects the wound made. While every veterinarian knows that hypodermic injections of medicine are daily made with a minimum of abscess production, it must be recognized that the conditions are not similar, and hence it is deemed highly advisable that these precautions be taken, especially in horses and mules.

There is usually little or no swelling at the point operated upon. Where abscess occurs the operator is to blame. It means that pus-producing germs have been carried in upon the needle and that either the needle or the skin was not disinfected. Abscess is more likely to occur in horses and mules than in other animals.

It has been the practice in Delaware to continue the vaccinated animals at their usual work. We have no data upon the subject showing this is unwise, but it is believed that the animals should be shielded as much as possible from excessive work and from extremes of heat or cold or from chilling rains. We advise our vaccinators, who consist of regular, practicing veterinarians designated by the governor upon the recommendation of the board of agriculture, to destroy all opened bottles of vaccine that remain unused at the end of a day's work, as it will certainly become contaminated and be spoiled if kept overnight at ordinary temperatures. The vaccine is dispensed in 50-dose bottles hermetically sealed and distinctively labeled, so there can be no mistake made in using it.

EFFECTIVENESS OF THE METHOD.

The results of vaccination have been as good, as shown by our statistics, as those obtained by the use of any other biological product. That the method is entirely safe is shown by the fact that only one dangerous swelling has been brought to our notice in three years' experience in Delaware. Every year there are instances where, for some reason, animals that have been vaccinated die from anthrax, showing that they were not protected by the vaccine. No doubt some of these failures may be due to the animal being missed during vaccinating. In other cases the failure may be due to the vaccine needle not properly puncturing the skin, so that the vaccine falls upon the ground. These accidents are readily brought about when the animals are unruly. On the other hand, the writer has observed a case of anthrax that occurred in vaccinated animals—that is, where it was positively certain that the vaccine was properly prepared and active and had been properly applied.

Some cases of nonprotection by vaccination can be explained by the fact that the vaccine itself was inert, and therefore nonprotective. Cultures made from such vaccine failed to grow. The vaccine would, of course, fail in the usual physiological test for an anthrax vaccine, and, of course, would fail to protect the animal against anthrax. The writer has known of instances where for various reasons an animal would escape vaccination, and that animal would be the only one to die on that farm. Hence he feels warranted in highly commending vaccination as the most important means of combating this terrible scourge. In France, where vaccination is most popular, and where statistics are reliable because of governmental control, the death rate before vaccination was adopted was, in cattle 5 per cent, and in sheep 10 per cent. After vaccination was adopted the losses were reduced to 0.34 per cent in cattle and 0.94 per cent in sheep. In Delaware the losses in 1907 in horses and cattle that were vaccinated were 0.32 per cent.

Vaccination should be practiced every spring, at least a month before it is time to turn the animals out to pasture, as a month is required for the production of immunity. This vaccinating should not be optional with the owner, as at present, but should be compulsory, the State assuming the risk of loss from the use of the vaccine, but not in those cases where it can be shown the animal died not from vaccination but from a natural infection, owing to failure of being protected by the vaccine, or from other causes.

A TEST OF PASTEUR VACCINES.

Along with the investigation of anthrax for the purpose of discovering new methods of treatment and prevention of the disease, and for studying the biology of the causative organism and the general sanitary aspects of the subject, it was decided to test the efficacy of freshly prepared Pasteur vaccines. It is believed that some of the bad results that have been reported as following the use of Pasteur vaccine is due to carelessness on the part of those preparing and using it. As there is no visible difference in the appearance of the two vaccines, Nos. 1 and 2, it is an easy matter to get the bottles mixed or improperly labeled. If the labels should come off the bottles, the vaccinator would have no guide as to which vaccine he was using. The writer has purchased anthrax vaccines on the open market and has found some that were wholly inert, and others that were too strong when tested in the usual way.

For the purpose of gaining such information as is possible from the practical experiences of practicing veterinarians who vaccinate against anthrax in Delaware, the writer undertook to prepare Pasteur vaccines for the State board of agriculture, which were to be used as soon as possible after they had reached destination. The cultures constituting the vaccines were grown in 50-dose bottles, each bottle containing 50 c. c. of bouillon which had been inoculated 24 hours previously with the vaccinal germs. The official vaccinator was furnished first with No. 1 vaccine. In 12 days he was shipped the No. 2 vaccine, for use on the same animals. Under this plan it was impossible for the bottles of vaccine to get mixed, even though the labels came off.

The plan of using fresh vaccine in which the germ is still in the bacillar stage was continued with good results for two seasons. For the last two years the plan of using vaccines that had been incubated for four or five days, or until the bacilli had spored, has been tried. This latter method represents the condition of vaccine as put on the market by commercial houses, and is a suspension of spores instead of bacilli. According to the experiences of these two seasons, we have reason to believe that the vaccine will remain active for several months, and such cultures may be prepared several months in advance of their use, provided the incubation is carried to a point where all growth ceases, or spores form. These spores inherit just that degree of strength possessed by their progenitors, and do not change except with a considerable lapse of time, possibly a year, if kept under favorable conditions. It is highly important that anaerobic conditions be not established in the bottles, as the bacilli will sink to the bottom and will not spore when grown anaerobically. To prevent this, it is important that the culture medium be made to reabsorb the air which has been driven out of it during sterilization before being inoculated with the vaccine germs. In preserving stock cultures of the vaccine it is very important that they be incubated for several days before being taken out of the incubator. If they are removed before spores have formed, the more vulnerable bacilli may succumb to existing unfavorable conditions. To these conditions may be ascribed the death of cultures of anthrax vaccine, and the loss of virulence of former virulent cultures of anthrax bacilli, which sometimes occur during the course of laboratory work.

EXPERIMENTS WITH VARIOUS SUBSTANCES TO TEST IMMUNIZING POWERS.

Experiments upon the disease have been carried on uninterruptedly for the past three years. These had for their object the preparation of substances in the laboratory and in living animals which could be used in combating anthrax by acting as antitoxins or bactericides, or as vaccines.

Realizing the great importance and economy in producing protective and curative substances in the laboratory over the necessarily expensive methods when employing animals for the same purposes, efforts have been made along the line of producing various culture products to be used in combating anthrax.

Pyocyanase, made after the method of Emmerich and Loew, gave some good results when tested upon rabbits, but failed upon sheep.

Anthraxase, prepared by the writer after the same general method used in producing pyocyanase, was without protective or curative properties, although it produced high fever when injected into rabbits and sheep subcutaneously.

Anthraxoin, consisting of a turbid suspension of dead anthrax bacilli, was apparently useless in protecting sheep against anthrax when used similarly to the Pasteur vaccine.

A single vaccine, having for its object the production of immunity in two weeks and thus cutting down the necessary period by one-half, was made by incubating a virulent bacillus for about 18 days at 42° to 43° C. Such a culture will kill guinea pigs in about a week, and in strength it thus holds a position between the two vaccines of Pasteur. With it sheep were vaccinated and after 12 days withstood an otherwise fatal infection with virulent bacilli. In some cases, however, the immunity was not sufficiently strong, as was evidenced by the death, now and then, of a sheep when tested with virulent bacilli.

The preparation and use of the various substances, together with the results of the experimental work, are described in the following pages.

ANTHRAXIN.

Anthraxin was made similarly to tuberculin and mallein. The cultures of anthrax were grown for 10 days with daily shaking in glycerinated, peptonized bouillon, the glycerin being used in 4 per cent strength. The cultures were then sterilized by boiling, filtered through Berkefeld filters, and then evaporated to one-tenth of the original volume. A sirupy liquid, much resembling tuberculin, resulted. It was tested on rabbits and sheep for immunizing properties, as shown in Table 1, on the following page.

Table 1.—*Experiments with anthraxin.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxin.	Virulent culture anthrax.		
	1907.			°*F.*	
Rabbit 1	July 10	1 c. c. subcutaneous.			
	July 17, 10 a. m.			105, 3 p. m.	
	July 18			104, 10 a. m.	
	do			103, 3 p. m.	
	July 19			Normal	
	July 24, 11 a. m.	1 c. c. subcutaneous.		104	
	July 24, 1.30 p. m.			105	
	July 24, 5.30 p. m.			105	
	July 25, 9 a. m.			Normal	
	July 31, 9 a. m.		0. 05 c. c.		
	Aug. 2, 9 a. m.				Dead from anthrax.
Sheep 1	Aug. 27, 1 p. m.	5 c. c. subcutaneous.			
	Aug. 27, 6 p. m.				Sheep blowing, head down.
	Aug. 28, 9 a. m.				Sheep seems well.
	Sept. 3, 9 a. m.	5 c. c. subcutaneous.			
	Sept. 10, 9 a. m.		0. 25 c. c.		
	Sept. 12, 9 a. m.				Dead from anthrax.
Sheep 2	Aug. 27, 1 p. m.	5 c. c. subcutaneous.			
	Aug. 27, 6 p. m.				Sheep blowing, head down.
	Aug. 28, 9 a. m.				Seems well.
	Sept. 3, 9 a. m.	5 c. c. subcutaneous.			
	Sept. 10, 9 a. m.	do			
	Sept. 23, 9 a. m.		0. 25 c. c.		
	Sept. 25, 9 a. m.				Dead from anthrax.
Sheep 3	Aug. 27, 9 a. m.	5 c. c. subcutaneous.			
	Aug. 27, 6 p. m.				Blowing; no fever.
	Sept. 3, 9 a. m.	5 c. c. subcutaneous.			
	Sept. 5				Diarrhea.
	Sept. 10, 9 a. m.	5 c. c. subcutaneous.			Well.
	Sept. 15, 9 a. m.		0. 25 c. c.		
	Sept. 17, 9 a. m.				Dead from anthrax.
Sheep 4	Aug. 27, 9 a. m.	5 c. c. subcutaneous.			
	Aug. 11, 6 p. m.				Blowing; no fever.
	Sept. 13, 9 a. m.	5 c. c. subcutaneous.			Diarrhea for several days.
	Sept. 10, 9 a. m.	do			
	Sept. 15, 9 a. m.		0. 25 c. c.		
	Sept. 17, 9 a. m.				Dead from anthrax.
Sheep 5 and 6	Sept. 3, 9 a. m.	5 c. c. subcutaneous.			
	Sept. 6				Seemed well.
	Sept. 10, 9 a. m.	5 c. c. subcutaneous.			
	Oct. 7, 9 a. m.		0. 25 c. c.		
	Oct. 9, 9 a. m.				Both dead from anthrax.

From the foregoing experiments we gather that anthraxin was possessed of no immunizing properties whatever.

PYOCYANASE.

Pyocyanase, the next substance experimented with, was prepared as follows: Large flasks of medium were inoculated with *Bacillus pyocyaneus* and grown at 37° C. until a ropy condition was produced, which required three weeks, the flasks meanwhile being shaken daily. The composition of the medium was as follows: Peptone, 0.5 per cent; glycerin, 0.1 per cent; dipotassium phosphate, 0.1 per cent; magnesium sulphate, 0.01 per cent; sodium chlorid, 0.3 per cent; sodium bicarbonate, 0.1 per cent; in distilled water (synthetic medium of Emmerich and Loew). When growth had ceased in this medium the growths from a number of agar-agar cultures of the same organism were added and the whole was thoroughly shaken. The culture medium was then neutralized with dilute hydrochloric acid. Carbolic acid was then added to 0.2 per cent strength as a preservative. The liquid was then evaporated down to one-tenth of its original volume at ordinary room temperature by being placed in pie plates. Then the liquid was dialyzed for 24 hours in running water, filtered through Berkefeld filters, and 0.2 per cent carbolic acid was again added to replace that which was dialyzed out. The resulting liquid, pyocyanase, has a dark coffee color and a pungent odor. The experiments to test its immunizing and curative properties upon guinea pigs, rabbits, and sheep are recorded in Tables 2 and 3.

TABLE 2.—*Experiments with unfiltered pyocyanase.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture anthrax.		
	1907.			°F.	
Rabbit 1	Dec. 9, 9 a. m.	6 c. c. subcutaneous.			
	Dec. 10, 9 a. m.		0.2 c. c. subcutaneous.		
	Dec. 11, 9 a. m.	4 c. c. subcutaneous.			
					Rabbit dead. Liver necrosed, spleen enlarged; anthrax bacilli at local lesion, but none found in blood or in liver and spleen; probably died from pyocyanase poisoning.
Guinea pig 1		3 c. c. subcutaneous.			
	Dec. 17, 10 a. m.		0.1 c. c. subcutaneous.		
	Dec. 18, 10 a. m.	1½ c. c. subcutaneous.			
	Dec. 18, 1.30 p. m.	2 c. c. subcutaneous.			Died during night. No bacilli in blood, but few at local lesion; probably died from pyocyanase poisoning.
Guinea pig 2	Dec. 16, 10 a. m.	3 c. c. subcutaneous.			
	Dec. 17, 10 a. m.		0.1 c. c. subcutaneous.		

TABLE 2.—*Experiments with unfiltered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture anthrax.		
	1907.			° F.	
Guinea pig 2	Dec. 18, 9 a. m.	1½ c. c. subcutaneous.			Sick.
	Dec. 19, 9 a. m.				Chloroformed. No bacilli in blood, but a few at local lesion; pyocyanase poisoning.
Guinea pig 3	Dec. 19, 9 a. m.	3 c. c. subcutaneous.			
	Dec. 20, 10 a. m.	do			
	Dec. 21, 3 p. m.	1½ c. c. subcutaneous.			
	1908.				
	Jan. 2, 9 a. m.		0.1 c. c. subcutaneous.		
	Jan. 4, 9 a. m.				Dead from anthrax. Bacilli in blood; another guinea pig treated similarly died of anthrax on third day after inoculation.
Rabbit 2	Jan. 13 9 a. m.		0.2 c. c. subcutaneous.		
	Jan. 14, 9 a. m.	6 c. c. subcutaneous.		103	
	Jan. 15, 9 a. m.	do		102	
	Jan. 16, 9 a. m.				Found dead of anthrax. Bacilli in blood.
Rabbit 3	Jan. 13, 9 a. m.	6 c. c. subcutaneous.			
	Jan. 14, 9 a. m.		0.3 c. c. subcutaneous.		Edema at points of injection of pyocyanase, no fever.
	Jan. 15, 9 a. m.	6 c. c. subcutaneous.		Normal.	
	Jan. 18, 9 a. m.				Animal apparently well.
	Jan. 25, 9 a. m.				Animal dies from anthrax on eleventh day. Bacilli in blood.
Rabbit 4	Jan. 13 9 a. m.	6 c. c. subcutaneous.	0.2 c. c. subcutaneous.		
	Jan. 14, 9 a. m.	do		Normal.	
	Jan. 15, 9 a. m.	do		do	
	Jan. 16, 9 a. m.				Dead from anthrax. Another rabbit treated similarly died of anthrax on third day also; bacilli in blood.
Rabbit 5	Feb. 3, 10 a. m.	½ c. c. subcutaneous.			
	Feb. 4, 10 a. m.	do	0.3 c. c. subcutaneous.	102.8	Slight swelling at point where pyocyanase was injected.
	Feb. 5, 10 a. m.	do			
	Feb. 8, 10 a. m.				Animal dead from anthrax, having lived four days.
Rabbit 6	Feb. 3, 10 a. m.	1 c. c. subcutaneous.			
	Feb. 4, 10 a. m.	do	0.3 c. c. subcutaneous.		Considerable reaction at point of injections.
	Feb. 5 10 a. m.	do			
	Feb. 7, 10 a. m.				Rabbit dead from anthrax on third day.
Rabbit 7	Feb. 3, 10 a. m.	2 c. c. subcutaneous.			
	Feb. 4, 10 a. m.	do	0.3 c. c. subcutaneous.	103	Small reaction from injections.
	Feb. 5, 10 a. m.			103	
	Feb. 8, 10 a. m.				Animal dead from anthrax, having lived four days.

TABLE 2.—*Experiments with unfiltered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture anthrax.		
	1908.			°F.	
Rabbit 8	Feb. 3, 10 a. m.	4 c. c. subcutaneous.			
	Feb. 4, 10 a. m.	1 c. c. subcutaneous.	0.3 c. c. subcutaneous.	103.5	Some reaction from injections. Abscess formed at point of injection.
	Feb. 7, 10 a. m.				Animal dead from anthrax.
Rabbit 9	Feb. 3, 10 a. m.	5 c. c. subcutaneous.			
	Feb. 4, 10 a. m.	2 c. c. subcutaneous.	0.2 c. c. subcutaneous.		Severe reaction at point of injection.
	Feb. 5, 10 a. m.			104	Abscess forms.
	Feb. 8, 10 a. m.			105	Animal dead from anthrax on fourth day.

The foregoing experiments were made with unfiltered pyocyanase. The dead bacilli produce abscesses at points of injection and frequently disease of the liver, which contributed to death from anthrax.

TABLE 3.—*Experiments with filtered pyocyanase.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture of anthrax.		
	1909.			°F.	
Sheep 1	Jan. 4, 10 a. m.	10 c. c. subcutaneous.		103.6	
	Jan. 4, 1 p. m.			105	
	Jan. 4, 3 p. m.	10 c. c. subcutaneous.	0.1 c. c. subcutaneous.	106	
	Jan. 5, 9 a. m.			103.6	Animal sick.
	Jan. 5, 4 p. m.			103	Off feed.
	Jan. 5, 7 p. m.				Dead from pyocyanase poisoning.
Sheep 2	Jan. 6, 9 a. m.	3 c. c. subcutaneous.		103	
	Jan. 6, 12 m.			105.5	
	Jan. 6, 4 p. m.			106.9	
	Jan. 7, 10 a. m.	1 c. c. subcutaneous.		104	
	Jan. 7, 11 a. m.				Respirations jerky.
	Jan. 21, 3 p. m.	5 c. c. subcutaneous.			
	Jan. 22, 9 a. m.	4 c. c. subcutaneous.		103.5	
	Jan. 22, 3 p. m.	2 c. c. subcutaneous.		102.5	
	Jan. 23, 9 a. m.	3 c. c. subcutaneous.		102.5	
	Jan. 23, 3 p. m.	2 c. c. subcutaneous.	0.1 c. c. subcutaneous.	103	
	Jan. 25, 9 a. m.			102	
	Jan. 26, 12 m.			102	
	Jan. 28, 10 a. m.				Dead from anthrax, having lived 5 days.
Sheep 3	Jan. 7, 10 a. m.	2 c. c. subcutaneous.		102	
	Jan. 7, 4 p. m.			107.2	Animal does not seem sick even though temperature is high.
	Jan. 13, 9 a. m.	2 c. c. subcutaneous.		101	
	Jan. 13, 3 p. m.	5 c. c. subcutaneous.	0.1 c. c. subcutaneous.	101	

TABLE 3.—*Experiments with filtered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture of anthrax.		
	1909.			°*F.*	
Sheep 3	Jan. 14, 9 a. m.	2 c. c. subcutaneous.		102.5	
	Jan. 14 1.30 p. m.			102.5	
	Jan. 15, 10 a. m.	2 c. c. subcutaneous.		104.5	Anthrax fever setting in.
	Jan. 16				Dead from anthrax (3 days).
Sheep 4	Jan. 8, 10 a. m.	1 c. c. subcutaneous.		101	
	Jan. 8, 1 p. m.			102	
	Jan. 8, 3 p. m.	1 c. c. subcutaneous.	0.1 c. c. subcutaneous.		
	Jan. 9, 10 a. m.	do		103	
	Jan. 10, 4.30 p. m.	do		103.5	
	Jan. 11, 9 a. m.			105.5	Dead two hours later of anthrax (3 days).
	1908				
Rabbit 10	Mar. 5, 9 a. m.	0.05 c. c. subcutaneous.		103.3	
	Mar. 6, 9 a. m.	0.5 c. c. subcutaneous.		103	
	Mar. 7, 9 a. m.	do			
	Mar. 8, 9 a. m.		0.2 c. c. subcutaneous.		
	Mar. 9, 9 a. m.	0.5 c. c. subcutaneous.		102.8	Rabbit well.
	Mar. 13, 9 a. m.				
	Mar. 18, 9 a. m.				Found dead of anthrax (10 days).
Rabbit 11	Mar. 5, 9 a. m.	1 c. c. subcutaneous.			
	Mar. 6, 9 a. m.	do		103	
	Mar. 7, 9 a. m.	do	0.2 c. c. subcutaneous.		
	Mar. 8, 9 a. m.			103	
	Mar. 10, 9 a. m.			101.5	
	Mar. 18, 9 a. m.	1.5 c. c. subcutaneous.		102.6	
	Mar. 21, 9 a. m.	3 c. c. subcutaneous.		103.7	
	Mar. 23, 9 a. m.	5 c. c. subcutaneous.		103	Animal sick.
	Mar. 24, 9 a. m.			103	Animal much better.
	Apr. 12, 9 a. m.				Much emaciated and swollen along belly.
	Apr. 13, 9 a. m.				Dead from anthrax. Post-mortem examination showed linear abscess in liver; repeated larger doses of pyocyanase probably caused animal to succumb to anthrax (lived 5 weeks); check rabbit died in 48 hours after inoculation.
Rabbit 12	Mar. 5, 9 a. m.	1.5 c. c. subcutaneous.		102	
	Mar. 6, 9 a. m.	do		102	
	Mar. 7, 9 a. m.	do	0.2 c. c. subcutaneous.	103	
	Mar. 8, 9 a. m.	do		102.8	
	Mar. 18, 9 a. m.	do		102.8	
	Mar. 20, 9 a. m.	3 c. c. subcutaneous.		103	
	Mar. 24, 9 a. m.	5 c. c. subcutaneous.		104.3	Animal has kept well.
	Apr. 12, 9 a. m.		0.1 c. c. subcutaneous.		
	Apr. 14, 9 a. m.				Animal found dead from anthrax, showing immunity was only passive (lived 24 days).
Rabbit 13	Mar. 18, 9 a. m.	5 c. c. subcutaneous.			
	Mar. 18, 2 p. m.	do	0.2 c. c. subcutaneous.		

TABLE 3.—*Experiments with filtered pyocyanase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Pyocyanase.	Virulent 24-hour culture of anthrax.		
	1908.			°*F*.	
Rabbit 13	Mar. 18, 4 p. m.			104	
	Mar. 19, 9 a. m.			104	
	Mar. 20, 9 a. m.	5 c. c. subcutaneous.		101	
	Mar. 28				Rabbit found dead from anthrax. Period of incubation lengthened to 10 days by pyocyanase; two more rabbits similarly treated reacted the same, and lived 10 days and 7 days, respectively.
Rabbit 14	Nov. 24, 9 a. m.	1 c. c. subcutaneous.			Simultaneous injections of "ase" and bacilli.
	Nov. 24, 3 p. m.	do	0.2 c. c. subcutaneous.	103	
	Dec. 4, 9 a. m.	do			
	Dec. 5.				
	Dec. 16	3 c. c. subcutaneous.			
	Feb. 4				Found dead from anthrax. Lived 21 days.
Rabbit 15	Nov. 24, 11 a. m.	2 c. c. subcutaneous.			
	Nov. 24, 3. p m.	do	0.2 c. c. subcutaneous.	104	
	Dec. 4, 9 a. m.	do			Some swelling which disappears in 2 days.
	Dec. 5				Animal has remained well up to Feb. 24, when it was killed by dog (lived 69 days).
	Dec. 16	3 c. c. subcutaneous.			
	Feb. 4				
Rabbit 16	Nov. 24, 11 a. m.	3 c. c. subcutaneous.			
	Nov. 24, 3 p. m.	do	0.2 c. c. subcutaneous.	104	Animal remains well.
	Dec. 4, 9 a. m.	do			
	Dec. 5, 9 a. m.			104.8	Swollen from "ase" injection.
	Dec. 16, 9 a. m.	3 c. c. subcutaneous.			
	Feb. 4				Animal has remained well up to Feb. 4, when it was killed by dog (lived 69 days). No lesions on post-mortem; check rabbit died in 48 hours after inoculation.
Rabbit 17	Nov. 24, 11 a. m.	4 c. c. subcutaneous.			Simultaneous injection.
	Nov. 24, 3 p. m.	do	0.2 c. c. subcutaneous.	104	Chloroformed to locate position and stage of development of bacilli. No spores or bacilli found by microscope or culture in local lesion, liver, spleen, or peritoneal cavity. Check rabbit died in 48 hours after inoculation.

From the foregoing experiments with pyocyanase it will be seen that when given in proper dose and simultaneously with virulent anthrax bacilli, the period of inoculation is greatly lengthened in rabbits. Sheep, however, seem to be very susceptible to poisoning by pyocyanase, and no immunity is conferred upon them by its use.

ANTHRAXASE.

Anthraxase was made in about the same manner as pyocyanase, except that the *Bacillus anthracis* was substituted for *Bacillus pyocyaneus*. The tests of this substance include cultures grown in ordinary bouillon, cultures grown in Emmerich and Loew's medium, and experiments with precipitated anthraxase. The details are given in Tables 4, 5, and 6.

TABLE 4.—*Experiments with anthraxase—Cultures grown in ordinary bouillon.*

Animal.	Date.	Injections.		Temperature.	Results and remarks.
		Anthraxase.	Anthrax culture.		
	1908.			°F.	
Rabbit 18	Mar. 17, 9 a. m.	0.75 c. c. subcutaneous.		101	
	Mar. 18, 9 a. m.	0.50 c. c. subcutaneous.		101.5	
	Mar. 19, 9 a. m.	do	0.2 c. c. subcutaneous.	102	
	Mar. 20, 9 a. m.	3 c. c. subcutaneous.		102.4	
	Mar. 21, 9 a. m.	5 c. c. subcutaneous.		107	Anthrax fever.
	Mar. 23, 9 a. m.	do			Animal very sick.
	Mar. 25, 9 a. m.				Dead from anthrax (lived 5 days).
Rabbit 19	Mar. 17, 9 a. m.	1.5 c. c. subcutaneous.		100.5	
	Mar. 18, 9 a. m.	do		102	Swollen lymphatics.
	Mar. 19, 9 a. m.	2 c. c. subcutaneous.	0.2 c. c. subcutaneous.	102.6	
	Mar. 20, 9 a. m.	3 c. c. subcutaneous.		104.6	Anthrax fever.
	Mar. 21, 9 a. m.				Dead from anthrax on second day.
Rabbit 20	Mar. 17, 9 a. m.	1 c. c. subcutaneous.		101	
	Mar. 18, 9 a. m.	do		101.5	
	Mar. 19, 9 a. m.		0.2 c. c. subcutaneous.		
	Mar. 20, 9 a. m.			104.1	Anthrax fever.
	Mar. 21, 9 a. m.	3 c. c. subcutaneous.		103	
	Mar. 22, 9 a. m.				Dead from anthrax on third day.
Rabbit 21	Mar. 23, 9 a. m.	0.5 c. c. subcutaneous.		103	Animal pregnant.
	Mar. 24, 9 a. m.	do		104	
				104	
	Mar. 26, 9 a. m.	5 c. c. intravenously.			
	do	3 c. c. subcutaneous.	0.2 c. c subcutaneous.		Animal died from shock.
Rabbit 22	Mar. 23, 9 a. m.	2 c. c. subcutaneous.		104.5	
	Mar. 24, 9 a. m.	do		104	
	Mar. 26, 9 a. m.	5 c. c. intravenously.	0.2 c. c. subcutaneous.	103.1	
	do	3 c. c. subcutaneous.			
	Mar. 27, 9 a. m.			102.9	
	Mar. 28, 9 a. m.	4 c. c. subcutaneous.		102.4	
	Mar. 30, 9 a. m.				Died of anthrax on fourth day.
Rabbit 23	Mar. 23, 9 a. m.	4 c. c. subcutaneous.		105	Animal excited.
	Mar. 24, 9 a. m.	do		106.5	
	Mar. 25, 9 a. m.				
	Mar. 26, 9 a. m.	5 c. c. subcutaneous.	0.2 c. c. subcutaneous.	103.4	

TABLE 4.—*Experiments with anthraxase, etc.*—Continued.

Animal.	Date.	Injections.		Temperature.	Results and remarks.
		Anthraxase.	Anthrax culture.		
	1908.			°*F.*	
Rabbit 23	Mar. 28. 9 a. m.	4 c. c. subcutaneous.			
	Mar. 30, 9 a. m.				Died of anthrax on third day.
Rabbit 24	Mar. 23, 9 a. m.	6 c. c. subcutaneous.		104.1	Animal pregnant.
	Mar. 24, 9 a. m.	do		105	
	Mar. 25, 9 a. m.			103.9	
	Mar. 26, 9 a. m.	5 c. c. intravenously.			
	do	3 c. c. subcutaneous.	0.2 c. c. subcutaneous.		
	Mar. 28, 9 a. m.	4 c. c. subcutaneous.		103.6	
	Mar. 30, 9 a. m.				Dead from anthrax on fourth day.
Rabbit 25	Mar. 25, 9 a. m.	3 c. c. intravenously.	0.1 c. c. subcutaneous.	103	Animal pregnant.
	Mar. 26, 9 a. m.	5 c. c. intravenously.		103.8	Much depressed by the injections and aborted at 4 p. m. (Control for this series dies in 48 hours after inoculation with the same culture.)
	do	3 c. c. subcutaneous.			
Rabbit 26	Apr. 7, 9 a. m.	0.5 c. c. subcutaneous.			
	Apr. 8, 9 a. m.	do		102	
	Apr. 9, 9 a. m.	do	0.1 c. c. subcutaneous.	103	
	Apr. 10, 9 a. m.	do			
	Apr. 11, 9 a. m.	2 c. c. subcutaneous.		105	
	Apr. 11, 4 p. m.	3 c. c. subcutaneous.		105.3	
	Apr. 12, 9 a. m.				Dead from anthrax on third day.

TABLE 5.—*Experiments with anthraxase. Cultures grown in Emmerich and Loew's medium.*

Animal.	Date.	Injections.		Temperature.	Results and remarks.
		Anthraxase.	Anthrax culture.		
	1908.			°*F.*	
Rabbit 27	Apr. 7, 10 a. m.	0.5 c. c. subcutaneous.		102	
	Apr. 9, 10 a. m.	do	0.2 c. c. subcutaneous.	102	
	Apr. 10, 10 a. m.	do		102	
	Apr. 11, 10 a. m.	1 c. c. subcutaneous.		102.3	
	Apr. 11, 4 p. m.	3 c. c. subcutaneous.		102.5	
	Apr. 12, 4 p. m.			103	Much swollen.
	Apr. 13, 4 p. m.				Dead from anthrax on fifth day.
Rabbit 28	Apr. 15, 10 a. m.	0.25 c. c. subcutaneous.			
	Apr. 16, 10 a. m.	do		100.8	
	Apr. 17, 10 a. m.	do		99.5	
	Apr. 18, 10 a. m.	do		99.8	
	Apr. 25, 10 a. m.	do	0.2 c. c. subcutaneous.	101	
	Apr. 26, 10 a. m.	0.50 c. c subcutaneous.		102.8	
	Apr. 27, 10 a. m.				Dies of anthrax in 2 days.

The following experiments on rabbits and sheep were made with precipitated anthraxase. Absolute alcohol, 9 volumes to 1 of anthraxase solution, was employed as the precipitant. The resulting yellow, gummy precipitate was readily soluble in water, to which 0.2 per cent strength of carbolic acid was added.

TABLE 6.—*Experiments with precipitated anthraxase.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxase.	Anthrax culture.		
	1908.			°*F.*	
Rabbit 29	Apr. 15, 9 a. m	0.5 c. c. subcutaneous.		101	
	Apr. 16, 9 a. m	do		101	
	Apr. 17, 9 a. m	do		101	
	Apr. 18, 9 a. m	do		101	
	Apr. 25, 9 a. m	do	0.01 c. c. subcutaneous.		
	Apr. 26, 9 a. m	do		101.8	
	Apr. 27, 9 a. m	do		102	Check rabbit dies.
	Apr. 27, 5 p. m	5 c. c. subcutaneous.			
	Apr. 28, 9 a. m			103	
	Apr. 29, 9 a. m			103	
	Apr. 30				Animal seems perfectly well.
	Nov. 5, 9 a. m		0.1 c. c. subcutaneous.		Given to test duration of immunity.
	Nov. 9, 9 a. m				Found dead of anthrax.
Rabbit 30	May 2, 10 a. m	1 c. c. subcutaneous.		103.3	
	May 2, 2 p. m	do		102.5	
	May 2, 4 p. m	do	0.1 c. c. subcutaneous.	103	
	May 4, 10 a. m	3 c. c. subcutaneous.		102	
	May 4, 4 p. m			104.5	
	May 5, 11.30 a. m	2 c. c. subcutaneous.		105	
	May 5, 4 p. m			104	
	May 6, 10 a. m			100.3	Dead of anthrax at 4 p. m. on fifth day.
Rabbit 31	May 2, 10 a. m	1 c. c. subcutaneous.		102.3	
	May 2, 2 p. m	do		101	
	May 2, 4 p. m	do	0.1 c. c. subcutaneous.	102	
	May 4, 10 a. m			104.8	Anthrax fever. Check dies to-day.
	May 5, 10 a. m	2 c. c. subcutaneous.		102.5	
	May 5, 4 p. m			101	
	May 6, 10 a. m			102	
	May 7, 10 a. m			102.5	
	May 8, 10 a. m				Animal turned out in yard.
	May 14, 10 a. m				Animal found dead. Postmortem showed death from anthrax and coccidiosis (lived 12 days).
Sheep 5	June 29, 10 a. m	5 c. c. subcutaneous.			
	June 29, 12 m	do			
	June 29, 2 p. m	do			
	June 29, 4 p. m	do	0.1 c. c. subcutaneous.		
	June 30, 10 a. m	do			
	June 30, 12 m	do			
	June 30, 2 p. m	do			
	June 30, 4 p. m	do			
	July 2, 9 a. m	do		105.8	
	July 2, 10 a. m	do		104.3	
	July 2, 12 m	do		105.8	Died of anthrax at 2 p. m. on fourth day.
Sheep 6	June 29, 10 a. m	do			
	June 29, 12 m	do			
	June 29, 2 p. m	do			

TABLE 6.—*Experiments with precipitated anthraxase*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxase.	Anthrax culture.		
	1908.			°F.	
Sheep 6	June 29, 4 p. m.	5 c. c. subcutaneous.	0.1 c. c. subcutaneous.		
	June 30, 10 a. m.	do.			
	June 30, 12 m.	do.			
	June 30, 2 p. m.	do.			
	June 30, 4 p. m.	do.			
	July 2, 8 a. m.	do.		102.2	Check guinea pig dies in 2 days.
	July 2, 10 a. m.	do.		102	
	July 2, 12 m.	do.		102	
	July 2, 3 p. m.	do.		102.8	
	July 2, 7 p. m.	do.		103	
	July 4, 10 a. m.	do.			Animal dead of anthrax on sixth day.
Sheep 7	July 28, 10 a. m.	do.			
	July 28, 12 m.	do.			
	July 28, 2 p. m.	do.			
	July 28, 4 p. m.	do.	0.1 c. c. subcutaneous.		
	July 29, 10 a. m.	do.		102.3	
	July 29, 12 m.	do.		102	
	July 29, 2 p. m.	do.		102.3	
	July 29, 4 p. m.	do.		102.6	
	July 30, 10 a. m.	do.		106	
	July 30, 12 m.	do.		106	
	July 30, 2 p. m.	do.		106	
	July 30, 4 p. m.	do.		106.2	
	July 31, 10 a. m.				Found dead of anthrax on fourth day after inoculation of test dose.

ANTHRAXOIN.

Anthraxoin was prepared and experimented with as follows: It consists essentially of a suspension, in carbolized normal salt solution, of dead, sporeless, anthrax bacilli. It was produced by inoculating, from a fresh anthrax carcass, bottles that had been filled completely with nutrient bouillon and plugged with hardest paraffin stoppers. In this way none but sporeless bacilli were introduced, and under the existing anaerobic conditions no spores could form. After all growth had ceased, at 35° C., these cultures were completely immersed in a water bath maintained at 55° C. for one hour, which was sufficient to devitalize the bacilli but not to destroy any antibodies they might contain. The dead bacilli were separated by filtration from the liquid in which they had grown, and those adhering to the Berkefeld filter were washed off in the requisite amount of carbolized normal salt solution. The suspension experimented with consisted of the bacilli that grew in 2,500 c. c. of bouillon, suspended in 50 c. c. of carbolized normal sodium chlorid solution. The details are given in Table 7.

TABLE 7.—*Experiments with anthraxoin.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxoin.	Anthrax culture.		
	1908.			°F.	
Sheep 8	Apr. 24, 10 a. m.	1 c. c. subcutaneous.			
	May 8, 10 a. m.	2 c. c. subcutaneous.			
	May 20, 10 a. m.		0.25 c. c. subcutaneous.		
	May 22, 10 a. m.				Sheep dies of anthrax in 2 days.
Sheep 9	Apr. 24, 10 a. m.	2 c. c. subcutaneous.			
	May 8, 10 a. m.		0.25 c. c. subcutaneous.		
	May 9, 10 a. m.			102.2	
	May 9, 4 p. m.			103	
	May 10, 10 a. m.			101.5	
	May 11, 10 a. m.			101.5	Remains well.
Sheep 10	Apr. 24, 10 a. m.	0.5 c. c. subcutaneous.			
	May 8, 10 a. m.	1 c. c. subcutaneous.			
	May 20, 10 a. m.		0.25 c. c. subcutaneous.		Sheep dies of anthrax in 3 days.
Sheep 11	May 11, 10 a. m.	1 c. c. subcutaneous.			
	May 24, 10 a. m.		0.25 c. c. subcutaneous.		Sheep dies of anthrax in 2 days.
Sheep 12	May 11, 10 a. m.	1 c. c. subcutaneous.			
	May 20, 10 a. m.		0.25 c. c. subcutaneous.		Sheep dies of anthrax in 3 days.
Sheep 13	May 11, 10 a. m.	1 c. c. subcutaneous.			
	May 23, 10 a. m.	5 c. c. subcutaneous.			
	June 8, 10 a. m.		0.25 c. c. subcutaneous.		
	June 10, 10 a. m.				Sheep dead from anthrax in 2 days.
Sheep 14	May 11, 10 a. m.	1 c. c. subcutaneous.			
	May 23, 10 a. m.	5 c. c. subcutaneous.			
	June 8, 10 a. m.		0.25 c. c. subcutaneous.		
	June 12, 10 a. m.				Sheep dead from anthrax in 4 days.
Sheep 15	Apr. 26, 9 a. m.	10 c. c. subcutaneous.		103	
	Apr. 26, 12 m.	do		104	
	Apr. 26, 2 p. m.	do		104	
	Apr. 26, 4 p. m.	do	0.25 c. c. subcutaneous.	104	
	Apr. 27, 9 a. m.	12 c. c. subcutaneous.		103	
	Apr. 27, 12 m.	do		103	
	Apr. 27, 2 p. m.	do		103.5	
	Apr. 27, 4 p. m.	do		103.5	
	Apr. 28, 9 a. m.	10 c. c. subcutaneous.		104	
	Apr. 28, 2 p. m.	do		103.5	
	Apr. 28, 4 p. m.	do		104	
	Apr. 29, 10 a. m.				Sheep dead from anthrax in 3 days.
Rabbit 32	Mar. 8, 10 a. m.	0.5 c. c. subcutaneous.			
	Mar. 8, 1 p. m.			104.1	
	Mar. 8, 4.30 p. m.			105.4	
	Mar. 9, 9 a. m.		0.2 c. c. subcutaneous.	101.8	

TABLE 7.—*Experiments with anthraxoin*—Continued.

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Anthraxoin.	Anthrax culture		
	1908.			°*F.*	
Rabbit 32	Mar. 10, 9 a. m			105.8	
	Mar. 11, 9 a. m				Dead from anthrax in 2 days.
Guinea pig	Mar. 8, 10 a. m	0.5 c. c. subcutaneous.			
	Mar. 9, 10 a. m		0.2 c. c. subcutaneous.		
	Mar. 11, 10 a. m				Dead from anthrax in 2 days.
Rabbit 33	Mar. 16, 9 a. m	0.1 c. c. subcutaneous.		101	
	Mar. 16, 11 a. m			102.2	
	Mar. 16, 12 m.			102.5	
	Mar. 16, 4.30 p. m.			104.3	
	Mar. 17, 9 a. m			101.5	
	Mar. 26, 9 a. m	0.1 c. c. subcutaneous.		102	
	Mar. 26, 4 p. m			103.5	
	Apr. 6, 9 a. m		0.01 c. c. subcutaneous.		
	Apr. 8, 9 a. m				Animal dead from anthrax in 2 days.
Rabbit 34	Mar. 16, 9 a. m	0.2 c. c. subcutaneous.		102	
	Mar. 16, 11 a. m			103.6	
	Mar. 16, 12 m.			104.5	
	Mar. 16, 1.30 p. m.			105.3	
	Mar. 17, 9 a. m			102	Rabbit stupid.
	Mar. 26, 9 a. m	0.2 c. c. subcutaneous.		102.5	
	Mar. 26, 2 p. m			105	
	Apr. 6, 9 a. m		0.01 c. c. subcutaneous.		
	Apr. 8, 9 a. m				Rabbit dead from anthrax in 2 days.
Rabbit 35	Mar. 16, 9 a. m	0.3 c. c. subcutaneous.		102	
	Mar. 16, 11 a. m			103.2	
	Mar. 16, 12 m.			103.5	Skin swollen at injection.
	Mar. 16, 2 p. m			104	
	Mar. 16, 4 p. m			105	Much swollen from injection.
	Mar. 17, 9 a. m			102.5	Swelling practically gone.
	Mar. 26, 9 a. m	0.3 c. c. subcutaneous.		102	
	Mar. 26, 4 p. m			104.5	
	Apr. 6, 9 a. m		0.01 c. c. subcutaneous.		
	Apr. 6, 9 a. m				Dead from anthrax in 2 days.

A COMMERCIAL VACCINE IN PILL FORM.

A commercial vaccine which, according to the makers, consists of dead anthrax organisms in pill form was also tested. These small pills are placed under the skin by means of a trocar and are claimed by the makers to produce immunity to anthrax. Microscopic examination, as well as cultural and animal experiments, show that the claims of the makers, in so far as the vaccine being dead and harmless is concerned, are true. One can easily see with the microscope that these little pills consist of dead anthrax bacilli and their spores held together in pill form by a proper excipient. The writer was unable, however, to verify the claim that they produce any immunity, as is shown in Table 2. A rabbit succumbed in six days, but as it is very rarely that a rabbit can be immunized by a vaccine, a sheep, which animal is easily protected, was also employed, with negative results.

TABLE 8.—*Experiments with commercial vaccine in pill form.*

Animal.	Date.	Injections.		Temperature.	Result and remarks.
		Vaccine.	Anthrax culture.		
	1908.			°*F.*	
Rabbit 36	Apr. 7, 9 a. m.	1 pill, subcutaneous.			
	Apr. 8, 9 a. m.	1 pill (dissolved), subcutaneous.			
	Apr. 9, 4 p. m.	do			
	do		0.01 c. c. subcutaneous.		
	Apr. 10, 4 p. m.	1 pill, subcutaneous.			
	Apr. 11, 4 p. m.			104	
	Apr. 13, 4 p. m.			104.3	
	Apr. 15, 4 p. m.				Dead from anthrax in 6 days.
Sheep 16	Oct. 17, 9 a. m.	1 pill with trocar.			
	Nov. 2, 9 a. m.		0.1 c. c. subcutaneous.		
	Nov. 4, 9 a. m.				Sheep dead from anthrax in 48 hours.

PREPARATION OF AN EFFECTIVE SINGLE VACCINE.

The single vaccine, like Pasteur vaccine, consists of cultures of attenuated anthrax bacilli, the only difference being in the degree of attenuation and that, as its name implies, it is applied only once, thus requiring a shorter time and only one handling of the animals.

To prepare such a vaccine or attenuated culture, the virulence of an already virulent culture of anthrax was exalted by passage through very young animals. A strain can in this way be produced which will kill a sucking rabbit in 24 hours. From the heart's blood of such a carcass tubes of bouillon are inoculated immediately after death. These cultures are then incubated at 42° to 43° C. for varying periods of time—from 12 to 18 days. On the twelfth day a subculture is made from one of the tubes and cultivated at 35° C. On the thirteenth and succeeding days subcultures are made from the remaining tubes until the series has been completed. These six subcultures of attenuated bacilli are now tested on guinea pigs and rabbits, and on sheep if possible.

More dependence is to be placed upon the animal test than upon the number of days the attenuation process is carried on. A culture of proper strength is generally obtained from tubes that have been attenuated for about 16 days. The proper culture will be one which will not kill rabbits, but which will kill a majority of guinea pigs in a delayed period, say 5 or 6 days. Such an animal should show no swelling at the point of inoculation, and the bacilli should be found only sparingly in the internal organs. When shaken in cultures such a vaccine shows a homogeneous clouding of the medium; no flocculi persisting, as occurs in virulent cultures.

As rabbits and guinea pigs can not be made immune to anthrax by vaccination of this kind, these animals are only useful in testing the pathogenesis of the cultures. For testing the immunizing property, sheep, which are easily immunized, were employed. Quite a number of sheep were used in the experiment to produce a safe, efficient single vaccine, and as regards the failures along this line, it is only necessary to say that they were entirely due to improper attenuation of the cultures. When the proper attenuation was reached there was no difficulty in immunizing animals by a single vaccination. This was effective against a subsequent, otherwise mortal dose of virulent anthrax bacilli, as the following experiments will show.

TESTS OF THE SINGLE VACCINE.

EXPERIMENT NO. 1.

On April 15, 1909, a visibly pregnant cow was given subcutaneously 1 c. c. of single vaccine. She showed no ill effects whatever from the injection. On May 1 the animal was inoculated with 0.2 c. c. of virulent anthrax bacilli from a 24-hour culture which killed a check rabbit and a check cow in 48 hours. On May 19 the cow dropped a fully developed calf which when found was dead, lying with its head bent under the shoulder. Supposing that the calf had been asphyxiated, it and the membranes were buried. However, a platinum loop full of the discharge was plated and a pure culture of 60,000 anthrax bacilli was obtained. A guinea pig inoculated with a culture made from one of these colonies died of anthrax in 48 hours. The cow remained well, and subsequent daily cultivations from vaginal discharges showed no anthrax bacilli.

This experiment was extremely valuable, not only as showing that the cow had been immunized by a single vaccination, but also for showing not only that anthrax can be communicated not only to the fetus, but that it can be thus communicated by an immune mother. It also showed that anthrax bacilli may persist for at least 18 days in the body of an immune animal. When the cow was destroyed for various reasons on May 25 she was in perfect health, and cultures and inoculations made with her blood showed no anthrax bacilli present. It is to be regretted that this animal could not have been kept for further observations and experiments.

EXPERIMENT No. 2.

On September 14, 1909, two sheep were vaccinated with single vaccine, each receiving 1 c. c., and showed no sickness therefrom. On September 28 each sheep was inoculated subcutaneously with 0.2 c. c. of a culture of anthrax bacilli whose virulence had been proven on a rabbit in the same experiment. The sheep at no time showed any sickness. On November 2 both sheep were again tested with virulent bacilli and showed no sickness.

EXPERIMENT No. 3.

On November 12, 1909, three sheep were vaccinated with 1 c. c. of single vaccine and showed no sickness therefrom. On November 27 one of the animals was tested with 0.2 c. c. of virulent bacilli, and on December 1 the other two were similarly tested. They at no time showed any sickness. These three sheep, together with the two used in experiment No. 2, were used later in experiments to produce an antibacterial serum.

A SERUM FOR PRODUCING IMMEDIATE IMMUNITY.

Although the favorable results from vaccination by the Pasteur system have been known for a long time, and owing to the cheapness of the vaccine it would seem that there is nothing more to be desired, the length of time required to produce immunity is one drawback in its use when one is endeavoring to check an existing outbreak of the disease. When vaccination is practiced a month in advance of the animals being turned out to pasture in the spring, the system of Pasteur vaccination is the proper one to use. In existing outbreaks, however, it is evident that any system that requires a month to become protective leaves much to be desired, as many animals could become infected and die before protection could be afforded them.

With the end in view of devising a method whereby an immediate immunity could be established in existing outbreaks or where an immunity could be brought about in a much shorter time than has been possible under the old system of vaccination, the writer has devoted a large portion of his time for the past three years. As it had been conclusively demonstrated that animals can be immunized by a single vaccine, thus cutting down one-half the period necessary for immunization by the old system of Pasteur, it was decided to experiment upon the production of an antianthrax serum by endeavoring to hyperimmunize sheep after the manner of the production of anti-hog-cholera serum, with the exception that whereas in the latter work virulent blood was used, this was precluded in our experiments owing to the dangers attending the handling of large quantities of virulent anthrax blood. Instead, the blood used was that drawn from an animal immune to anthrax, and not from one sick with anthrax.

Briefly stated, the writer has produced an antibacterial serum by highly immunizing sheep through repeated inoculations, first of attenuated anthrax bacilli, and following these by inoculations of the most virulent races of the bacilli in increasing doses until the animal would withstand with impunity fifty thousand times the minimal lethal dose. Such a serum will protect a sheep against an otherwise mortal dose of bacilli and produce an immediate immunity. It is, therefore, a very valuable adjunct in working against the spread of the disease in existing outbreaks where the usual vaccination is being practiced. The serum will confer a passive immunity immediately, and thus protect the animal against fatal infection until the vaccine confers an active immunity. And should an animal which has been protected by the serum become infected with a virulent anthrax bacillus, the results of this infection will be the production of a much stronger immunity than the vaccine and serum would otherwise confer.

It is evident that if a single vaccine, or even a double vaccine, can be used in conjunction with an antibacterial serum to produce immediate passive immunity which will persist until the vaccine has had time to bring about active immunity a long step will have been made in controlling this formidable disease. The experiments with this end in view were carried out upon sheep as follows:

EXPERIMENTS WITH THE SERUM.

Experiment No. 1.

On December 15, 1909, a sheep immunized with single vaccine and a subsequent inoculation with virulent bacilli (see Experiments Nos. 2 and 3 with single vaccine) was bled from the carotid artery—the femoral artery being small and deep-seated—by means of a glass cannula and rubber tubing. When the blood had clotted, 500 c. c. of serum were decanted and injected into the inguinal regions of another sheep which had been similarly immunized. Microscopic examinations and inoculations into guinea pigs of this blood showed it to be free of anthrax bacilli. The injected sheep was lame in both hind legs the next day, but this lameness disappeared when the complete absorption of the injected blood had taken place, and no abscess formed. On January 12 this sheep was bled from the carotid artery, and when the serum was collected it was preserved by adding to it one part of a 5 per cent solution of carbolic acid to each nine parts of serum. In making this addition of carbolic acid some coagulation will occur if the preservative and serum be not poured simultaneously into another vessel and stirred by a helper.

The above serum was without protective properties. All animals upon which it was used died of anthrax, as follows:

On January 13, 1910, 12 guinea pigs received increasing doses of serum from 0.1 up to 3 c. c. and simultaneously 0.1 c. c. of virulent anthrax culture. All of these animals died of anthrax in 48 hours.

On January 17, 1910, the amounts of serum were increased to 4 c. c., 5 c. c., 6 c. c., 7 c. c., 8 c. c., 9 c. c., and 10 c. c., with a simultaneous dose of 0.1 c. c. of virulent culture. The 7 animals thus tested also died in 48 hours.

On February 5, 1910, a sheep received subcutaneously 24 c. c. of the serum and 0.2 c. c. of virulent culture. A control rabbit died in 48 hours and the sheep died of anthrax in three days.

On February 9, 10, and 11 another sheep received daily 12 c. c. of the serum and on the 12th a test dose of virulent bacilli. This sheep lived four days and then died of anthrax.

These experiments demonstrated that the modified hog-cholera method of serum production could not be applied to anthrax, and it was then decided to try and bring about hyperimmunization by increasing doses of virulent bacilli repeated over a long period. The two sheep used in experiment No. 2 with single vaccine were employed for this purpose as follows:

EXPERIMENT No. 2.

On September 14, 1909, two sheep were vaccinated with 1 c. c. of single vaccine. On September 28 they were given a test dose of 0.2 c. c. of virulent bacilli, and remained well, while a check rabbit died in 48 hours. On November 2 each sheep again received 0.2 c. c. of virulent bacilli. On March 4, 1910, each sheep received 0.3 c. c. of virulent bacilli. On March 18 they received 1 c. c. of virulent bacilli, and on April 2 each sheep received 5 c. c. of virulent bacilli. A period of about seven months was thus consumed.

On April 14, 1910, these sheep were bled from the carotid artery, producing 1,900 c. c. of serum, to which was added one part of a 5 per cent solution of carbolic acid to each nine parts of serum as a preservative. Prior to the addition of the preservative tests for the presence of anthrax bacilli were made upon guinea pigs and by plate cultures. None were found.

This serum was used upon sheep in connection with virulent anthrax bacilli as shown in Table 9.

TABLE 9.—*Experiments with antianthrax serum.*

Animal.	Date.	Injections.		Results and remarks.
		Immune serum.	Virulent culture.	
	1910.			
Sheep 17	Apr. 29	5 c.c. subcutaneous	0.2 c. c. subcutaneous	
	May 16		.2 c. c. subcutaneous	Sheep remains well.
Rabbit (control)	do		.1 c. c. subcutaneous	Rabbit dies in 48 hours.
Sheep 18	May 21	10 c. c. subcutaneous.	.1 c. c. subcutaneous	Not sick.
	June 20	do	.2 c. c. subcutaneous	No bad result.
	July 6	do	.5 c. c. subcutaneous	Remained well.
Rabbit (control)	May 21		.1 c. c. virulent culture.	Died in 48 hours.
Sheep 19	May 24	10 c. c. subcutaneous.	.1 c. c. subcutaneous	
	June 20	do	.1 c. c. subcutaneous	Remained well.
	July 6	do	.5 c. c. subcutaneous	
Rabbit (control)	May 24		.1 c. c. virulent culture.	Died in 48 hours.
Sheep 20	May 27		.5 c. c. single vaccine	
	June 20	10 c. c. subcutaneous.	.1 c. c. virulent culture.	
	July 6	do	.5 c. c. virulent culture.	Remained well.
Sheep 21	May 27		.5 c. c. single vaccine	
	June 20	10 c. c. subcutaneous.	.1 c. c. virulent culture.	Do.
	July 6	do	.5 c. c. virulent culture.	
Rabbit (control)	June 20		.1 c. c. virulent culture.	Dead in 48 hours.
Sheep 22	June 1	5 c. c. subcutaneous.	1.0 c. c. single vaccine	
	June 18	10 c. c. subcutaneous.	.1 c. c. virulent culture.	Remained well.
	July 6	do	.5 c. c. virulent culture.	
Sheep 23	June 1	5 c. c. subcutaneous	1.0 c. c. single vaccine	
	June 18	10 c. c. subcutaneous.	.5 c. c. virulent culture.	Do.
	July 6	do	.5 c. c. virulent culture.	
Sheep 24	June 1	5 c. c. subcutaneous	1.0 c. c. single vaccine	
	June 18	10 c. c. subcutaneous.	.1 c. c. virulent culture.	Do.
	July 6	do	.5 c. c. virulent culture.	
Sheep 25 (control).	June 18		.1 c. c. virulent culture.	Died of anthrax in 48 hours (control for sheep 22, 23, and 24, for inoculations June 18).
Rabbit (control)	July 6		.1 c. c. virulent culture.	Died of anthrax in 48 hours (control for sheep 22, 23, and 24, for inoculations on July 6.

No doubt the period of time occupied in hyperimmunizing the sheep which produced this highly protective serum can be very much lessened—probably one-half. It will be noted that the time covered was about seven months. This was not intentional, but as the sheep had already been immunized for another object, and not used, it was decided to carry out the idea of greatly increasing their immunity by the method followed. It will be seen by referring to the table that an idle period of about three months elapsed between November, 1909, and February, 1910, which might just as well have been employed for the purpose. It is also possible that by giving larger doses of virulent bacilli the immunizing property of the serum may be considerably increased.

That the degree of immunity can be established by the number of inoculations and the quantity of virulent culture used was clearly shown in sheep Nos. 18, 19, 20, 21, 22, 23, and 24. They had received three injections of serum and three inoculations with bacilli, in very small doses, and while they themselves were immune (see Table 9) their blood, when tested after slaughter on July 18, did not protect other sheep.

It is also highly probable that sheep will produce a more effective antianthrax serum for their own species, and cattle for cattle, horses for horses, etc.; that is, the sera should be homologous. However, no effort was made to test this principle, as the great expense attending such experiments precluded their being carried out.

CONCLUSION.

The writer does not advise the abandonment of the old Pasteur system of vaccination against anthrax when it is practiced upon animals before they are turned out on the pastures in the spring of the year. When animals are dying, however, vaccination alone requires too long a period to protect, and it is in these outbreaks that the antianthrax serum should be used in conjunction with vaccine. The experiments have shown that a single vaccine may be used with good results. Where it is desired, however, the serum may be used simultaneously with the double vaccine or with a single vaccine.

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636
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THE MILK SUPPLY
OF
CHICAGO AND WASHINGTON.

BY

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., April 5, 1911.

Sir: I have the honor to transmit the accompanying report on The Milk Supply of Chicago and Washington, prepared by Dr. George M. Whitaker, of the Dairy Division of this bureau, and to recommend its publication in the bureau's bulletin series.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

PART I.—THE MILK SUPPLY OF CHICAGO.

Page.
Amount of the supply 7
Source of the supply 8
Transportation and freight 11
  Freight rates 12
Methods of handling the milk 13
  Methods of the large dealers—"country bottling" 13
  Methods of the small dealers 16
  Handling of milk in stores 17
  Brokerage 18
Organizations 19
  Producers' organizations 19
  Dealers' organizations 20
  The milk drivers' union 20
Wholesale and retail prices 20
The official inspection 24
  Existing conditions 27
  Infant mortality 29
The production of high-grade milk 29

PART II.—THE MILK SUPPLY OF WASHINGTON.

Amount and source of the supply 31
Transportation and freight 31
  Freight rates 33
The producers 33
Methods of handling milk in the city 34
Dealers' and producers' organizations 35
Wholesale and retail prices 35
Laws and inspection 35
  A milk-standard decision 39
Federal cooperation 39

ILLUSTRATIONS.

PART I.—CHICAGO.

PLATES.

Page.

PLATE I. General view of a high-class Chicago milk-bottling plant............ 14
II. Interior views of plant shown in Plate I........................... 14
III. Undesirable Chicago milk plants................................. 16
IV. Chicago city milk plants of the better class........................ 16

TEXT FIGURES.

FIGURE 1. Map showing source of greater part of Chicago's milk supply........ 9
2. Map showing location of country milk-bottling plants supplying Chicago.. 15
3. Monthly range of wholesale milk prices at Chicago................ 22
4. Summer and winter wholesale milk prices at Chicago............. 23

PART II.—WASHINGTON.

PLATES.

PLATE V. Handling milk for Washington.. 32
VI. Unloading milk at railroad stations in Washington.................. 32
VII. Undesirable Washington milk plants.............................. 34
VIII. Views in high-class Washington milk plants......................... 34

TEXT FIGURES.

FIGURE 5. Map showing source of most of Washington's milk supply........... 32
6. Chart showing decrease in death rate from diarrhea and enteritis in children under 2 years of age in the District of Columbia, following enactment of milk law of 1895.................................. 38

THE MILK SUPPLY OF CHICAGO AND WASHINGTON.

Part I.—THE MILK SUPPLY OF CHICAGO.

AMOUNT OF THE SUPPLY.

Exact figures as to the amount of milk consumed in Chicago are difficult to obtain because of the peculiar reticence of the railroads regarding the amount of milk transported by them. Fortunately a similar condition does not exist in other large cities. One of the best examples is Boston, where the railroads make monthly reports to the State railroad commissioners of the amounts of milk shipped into the city. These figures are then furnished by the commissioners to the newspapers and other interested parties. In Chicago none of the railroads make any such reports; some absolutely refuse information, even to an official inquirer, and some make partial or guarded statements on the pledge that no use shall be made of them except in general compilations.

But though official figures are lacking, some estimates can be made from which an approximation of the amount of milk used in Chicago can be reached. It has been estimated that the average daily per capita consumption of milk throughout the United States is 0.6 to 0.65 of a pint.[1] Applying the larger figure to 2,500,000 people gives 812,000 quarts a day. The consumption of milk according to all records available is greater in the North than in the South; hence it is fair to assume that the consumption in a northern city is above the average. It is also fair to assume that the transient hotel population of a large metropolis like Chicago would materially swell the consumption. In 526 replies to 2,000 cards mailed to physicians, lawyers, business men, and laborers in Chicago by the Dairy Division the writers reported on an average a per capita consumption in their own families of 1 pint a day. That rate carried through the whole population would give as the total consumption 1,250,000 quarts. This figure is probably too large, as 800,000 is too small.

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[1] Henry E. Alvord, Statistics of the Dairy, Bulletin 55, Bureau of Animal Industry, p. 48, 1903.

Various local authorities estimate the daily railroad receipts at between 25,000 and 30,000 cans, each holding 32 quarts. The health department in 1910[1] estimated the daily milk receipts at 31,245 32-quart cans. This would be about 1,000,000 quarts. Probably the daily consumption of milk is not far from this amount. The health commissioner estimated that 152,000 cows furnished the city's milk supply in 1908, which would make the average production 6.3 quarts per day per cow. The census of 1900 estimates the average yield of milk for each cow in Illinois at 5 quarts a day, and there is no doubt that the cows now producing milk for Chicago do better than the average for the State in 1900. His estimate for 1910 was 120,000 cows.

SOURCE OF THE SUPPLY.

Most of the milk supply of Chicago is produced within 60 miles of the city, and a 100-mile circle about the city would include nearly all of the dairies producing its supply, though in times of exceptional scarcity in the summer sweet cream is shipped 200 miles. The production of Chicago's milk within such a short distance of the city is in marked contrast with conditions in Boston and New York. Greater Boston has a population only one-third that of Chicago, but most of its milk supply is produced outside of a 50-mile circle, and some milk cars start 200 miles from the city.[2] New York City, with a million more population than Chicago, receives practically no milk from within 50 miles, and some of its supply comes from points as far away as 400 miles.

The Chicago milk supply is chiefly produced in 24 counties, of which 11 are in Illinois, 8 in Indiana, and 5 in Wisconsin (see fig. 1). Occasionally a little milk comes from Michigan. Fully three-fourths of the total supply is produced in Illinois.

Another peculiarity of the source of the supply of Chicago milk is that production has extended to the northwest much more than in other directions, and is not evenly distributed over the milk-producing territory, but is largely bunched in two counties in northern Illinois, which more than equal the combined production of the other nine milk-producing counties of that State.

McHenry County, Ill., is the third largest market milk producing county in the United States, being credited in the census of 1900 with sales of 29,194,198 gallons. It is excelled only by St. Lawrence and Orange Counties, N. Y., with sales of 39,795,642 and 29,617,072 gallons, respectively. Kane County is the second milk-producing county in Illinois and the fourth in the United States, with 24,244,532 gallons. The significance of these figures will be more fully appreciated when it is realized that according to the 1900 census there were only eight counties in the whole United States where the sales were in excess of 20,000,000 gallons.

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[1] Report of the Chicago Board of Health, 1907-1910, p. 317.

[2] The Milk Supply of Boston, New York, and Philadelphia, Bulletin 81, Bureau of Animal Industry. pp. 8, 41, 1905.

That the milk supply of a city as large as Chicago is produced so near the place of consumption is somewhat remarkable, especially when we remember that its lakeside location cuts off the possibility of any milk production to the east and much of the north and reduces the field of supplies to the western and southerly sections.

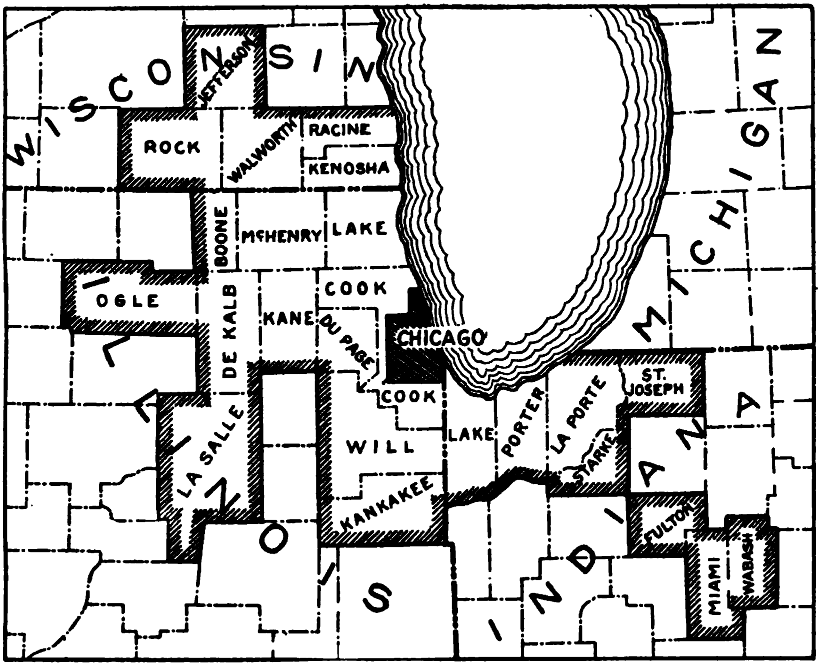

FIG. 1.—Map showing source of greater part of Chicago's milk supply.

In spite of these conditions there is one reason for so large a production of milk comparatively near the city, namely, the fact that Chicago is not surrounded by a circle of suburban towns and cities or by large areas of land used by city people for rural residences and held at comparatively high building-lot figures. As compared with New York, Philadelphia, and Boston there is in the case of Chicago a somewhat abrupt transition from the thickly settled portion of the city to farming land. This is true to such an extent that some milk cars take on milk within 15 miles of the city, and there are two milk-shipping stations in Cook County, where Chicago is located, which sold 14,500,000 gallons during the census year 1900. So much territory has been annexed to develop the "greater Chicago" idea that some dairying is carried on within the city limits, about 1,400 cows being kept for this purpose.

The extraordinary development of production to the northwest is largely due to the energetic pushing work of the railroads running through that section in securing shipments of milk. The possibly accidental locating in this region years ago of some dealers who have become the largest in the locality may have been another element. Land values and dominating crops may also have had an influence. Traveling southerly from Chicago, one reaches in hardly more than an hour vast stretches of level fertile land admirably adapted to dairying, but largely devoted to corn. This land is so valuable that it is considered by many farmers as more profitable for corn raising than for milk production. The assertion is frequently heard that a farmer can not afford to produce milk on such high-priced land. The extension of the milk-producing territory to the northwest has carried it into the southern tier of Wisconsin counties. More recently, however, the business has been pushed in a southeasterly direction into Indiana, and most of the increase of late years has been in that direction. There has not been much extension of milk mileage in Illinois for several years.

From 1906 to 1908 milk was rather scarce. During the winter of 1908–9 there was an oversupply, attributed in part to the number of people out of employment, in part to the cry of "trust," many persons refusing to help fill the coffers of those whom they regarded as extortionists, and in part to the talk about unclean milk due to the agitation for more rigid inspection. This oversupply continued into the early summer of 1909, when a severe and continued drought curtailed the supply and caused an uncomfortable scarcity.

During the fall and winter of 1909 and the spring of 1910 the milk supply was normal, with no excessive shortage or surplus, but the drought in July and August created a record-breaking shortage, to be followed by a comfortable ratio between supply and demand during the winter of 1910–11, which was a fairly prosperous one for the producers, one authority calling it "the most profitable period in local history."

During the fall of 1910 the increased use of condensed milk in other places proved a benefit to the producers for the local market by creating a greater demand for their product, which made considerable inroads on the market milk supply and restricted the surplus.

TRANSPORTATION AND FREIGHT.

In spite of the aversion of the railroads to stating the amount of their milk shipments, the report of the Chicago health department for 1907–1910, page 317, publishes the following:

Number of 8-gallon cans of milk delivered in Chicago by various railroads, 1898–1910.

Railroads.	1898	1899	1900	1901	1902	1903	1904
Chicago & North Western	5,400	5,400	6,000	6,500	8,500	9,000	10,000
Chicago, Milwaukee & St. Paul	2,600	2,660	3,500	4,000	5,500	5,500	6,500
Illinois Central	1,500	1,550	1,500	1,900	2,300	2,300	2,500
Chicago Great Western	1,600	1,600	1,200	1,550	1,700	1,700	1,800
Wisconsin Central	800	800	700	1,000	1,000	1,000	1,000
Chicago, Burlington & Quincy	800	800	700	900	950	1,000	1,000
Atchison, Topeka & Santa Fe	550	550	500	600	650	600	600
Grand Trunk	450	450	400	500	500	500	500
Rock Island	450	450	500	700	600	500	500
Erie	400	400	300	400	450	500	500
Wabash	350	350	300	420	400	400	400
Pennsylvania	300	350	400	550	300	300	300
Pittsburg, Cincinnati, Chicago & St. Louis	200	200	....	....	300	300	300
Monon	150	150	200	200	250	300	300
Chicago & Eastern Illinois	200	200	200	250	300	300	300
Baltimore & Ohio	250	250	200	300	300	300	300
Chicago & Alton	100	100	200	250	300	300	200
Aurora, Elgin & Chicago Electric	....	....	....	....	....	....	200
Bottled milk	....	1,300	3,325	4,130	....	....	....
Total	16,100	17,560	20,125	24,150	24,300	24,800	27,200

Railroads.	1905	1906	1907	1908	1909	1910
Chicago & North Western	10,000	11,000	11,000	11,000	12,000	12,200
Chicago, Milwaukee & St. Paul	6,600	6,500	6,500	7,500	8,000	8,600
Illinois Central	2,600	2,600	2,600	2,600	1,865	1,775
Chicago Great Western	1,800	1,700	1,700	1,700	1,175	1,400
Wisconsin Central	1,600	1,800	1,800	1,800	940	800
Chicago, Burlington & Quincy	1,100	1,000	1,000	1,000	815	800
Atchison, Topeka & Santa Fe	600	600	600	600	500	300
Grand Trunk	550	500	500	500	520	500
Rock Island	500	500	500	500	580	600
Erie	500	500	500	500	550	620
Wabash	400	400	400	400	200	300
Pennsylvania	400	550	600	600	475	500
Pittsburg, Cincinnati, Chicago & St. Louis	400	400	400	400	200	....
Monon	400	400	500	500	200	300
Chicago & Eastern Illinois	350	400	400	400	60	25
Baltimore & Ohio	300	300	300	300	425	480
Chicago & Alton	300	300	300	300	315	200
Aurora, Elgin & Chicago Electric	350	400	300	300	600	600
Cows inside city limits	....	....	....	....	500	700
Hauled by wagon	....	....	....	....	230	400
Miscellaneous	....	....	....	....	105	145
Total	28,650	29,850	30,000	30,000	30,260	31,245

From this it will be seen that the milk supply of the city comes in on about 18 different railroads, but nearly two-thirds of it comes over two lines entering from the northwestern territory previously noted. The relative amount of shipments over the different leading lines is as follows:

	Per cent.
Chicago & Northwestern	39
Chicago, Milwaukee & St. Paul	26
Illinois Central	5
Chicago Great Western	4
Wisconsin Central	2
Chicago, Burlington & Quincy	2
Others	22
	100

The milk supply has steadily increased from year to year with the growth of the city, and there has been a corresponding increase of milk shipments by the leading roads without much change in their relative standing. The roads doing the largest business have not increased their milk mileage with the larger shipments. The chief change has been in a reduction of the number of individual shippers and an increase of business from the bottling plants.

The large dealers who ship bottled milk by the carload ice it in the summer, but the supply of can milk comes in ordinary baggage cars, largely on passenger trains. It is in transit such a short time that no efforts at refrigeration are made. A single car of milk in cans may contain supplies for 50 or more dealers. A city ordinance requires this milk to come into the city in sealed cans.

FREIGHT RATES.

There is no uniformity in the system of freight rates for milk on the different railroads and no regular zone system of charges as in New York and Boston.[1] The rates of each road are independent of all others. Most of the roads issue a sheet giving a rate for each of the different shipping stations on their lines. When this is worked out on the basis of the distance of those stations from the city we get the following results on three of the roads, the price referring to a can of 32 quarts:

(1) Less than 25 miles, 12 cents; 25 to 50 miles, 15 cents; 50 to 75 miles, 20 cents.

(2) Less than 36 miles, 15 cents; 36 to 42 miles on main line and to 49 miles on branches, 16 cents; 42 to 46 miles on main line, 17 cents; 52 to 59 miles, 18 cents; 62 to 66 miles, 19 cents; 68 to 83 miles, 20 cents.

(3) Less than 10 miles, 14½ cents; 10 to 21 miles, 15 cents; 21 to 30 miles, 15½ cents; 32 to 55 miles, 16 cents; 55 to 62 miles, 17½ cents.

Stated in a general way, the matter resolves itself practically into three zones, in which the freight rate is 15, 16, and 16½ cents a can for much of the milk shipped. Sixteen cents a can is half a cent a quart, and each extra cent per can for additional distances adds three one-hundredths of a cent per quart.

As regards bottled milk in carload lots there is no official carload, but the rate is based on at least 300 crates to the car at 10 to 14½ cents a crate, or a minimum of $20 per day per car. The dealers estimate the freight to cost them on an average three-fourths of a cent per quart. The rates are the same for milk and cream. Much milk is on the road only two or three hours.

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[1] Bulletin 81, Bureau of Animal Industry, pp. 11, 42.

METHODS OF HANDLING THE MILK.

Comparatively little milk is distributed in Chicago by the producers. Almost the entire product passes through the hands of middlemen. Of these there are two distinct types, employing distinct methods, and it happens that as a rule the larger dealers do business in one way and the smaller dealers in another. Hence, the line between the large and small dealers is more sharply drawn in Chicago than in most other cities, being a matter both of relative magnitude of business done and of methods employed.

METHODS OF THE LARGE DEALERS—"COUNTRY BOTTLING."

Some eight to twelve of the larger milk dealers in Chicago do about one-half the business of the city, and of these two concerns stand out preeminent, having about two-thirds of the business that is done by the larger dealers. This estimate would give one-third of the city's milk business to these two dealers. The large city dealers receive their supplies from the producers at country stations which the dealers own and operate. On account of the fact that the milk from these places is sold in bottles, filled at the stations, local usage has given the name of "bottling plants" to the milk-receiving depots, rather than "shipping stations" or "creameries," names used in other States for places having a similar use. These stations are located near a railroad and connected with it by a spur track. In the morning the farmers deliver at the station the milk of that morning and the previous evening. It is as a rule cooled by the farmers to 60° F. or below, in tanks of ice or well water, mostly the latter. It is usually delivered in 32-quart cans owned by the farmers. Every morning one will see near these milk stations long lines of farmers' wagons on the road leading to the receiving platform of the station, waiting to unload. Ordinary farm spring wagons are used, and the cans are covered with canvas.

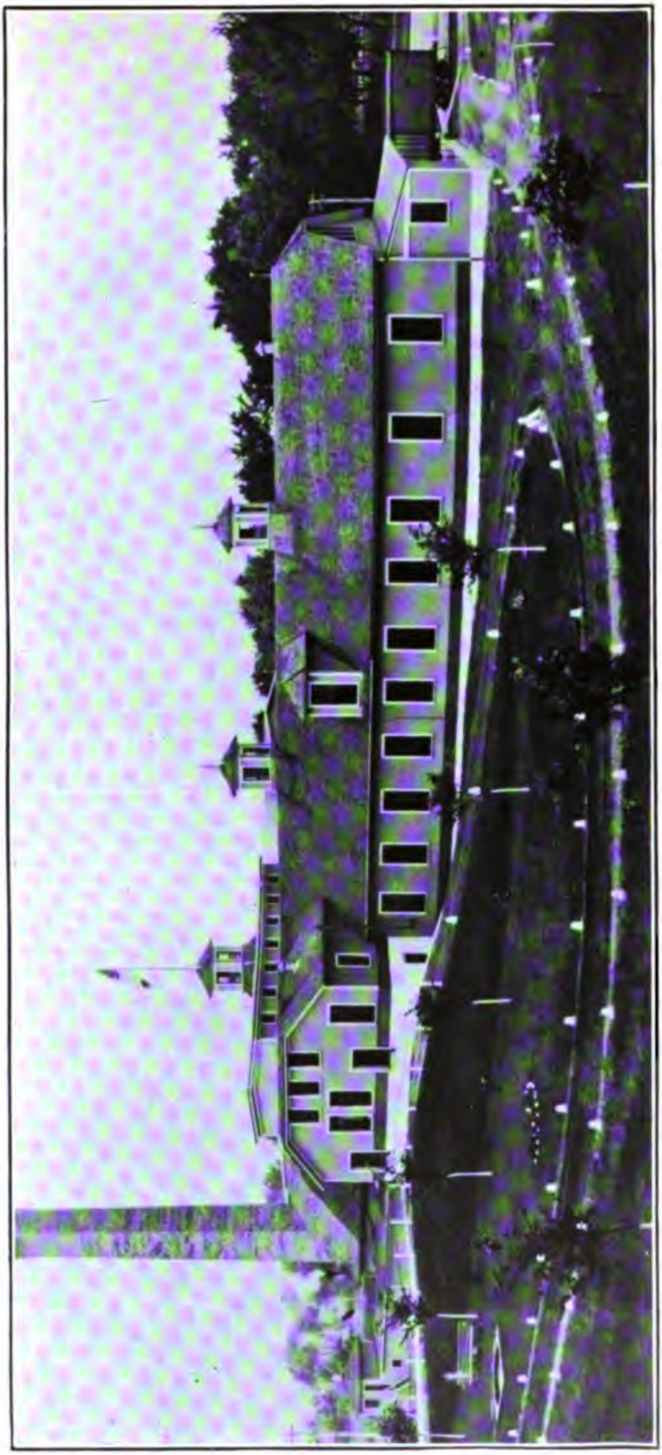
When a farmer's cans have been emptied into the weight vat in the receiving room the cans are passed to an adjoining room where they are washed and steamed while the farmer waits. In a few minutes after delivering his load he is on his way home with clean and steamed cans. He has also received credit in the weigh room for the amount of his delivery. Milk handled at these stations is bought by the hundredweight. No milk is bought on the basis of fat percentage, though there is a minimum requirement. Usually each farmer delivers his own product at the station, but occasionally the producers cooperate in the hauling; in such cases it is customary for each one of a small group to take turns in hauling his milk and that of his neighbors to the station. Most of the producers live within 4 miles of their stations, though in extreme cases one is found 5 to 5½ miles distant.

As soon as the milk is weighed it goes to the milk rooms, where it is mixed, cooled, and bottled. Some of these "bottling plants" filter, clarify, and pasteurize the milk as well. When bottled it is crated and the crates are iced for transportation. Some plants have a condensing outfit and do a large condensed milk business in addition to their ordinary market milk trade. Many plants have separators for obtaining cream for the cream trade, and most of them are equipped for manufacturing butter in case of a surplus of milk and also for making buttermilk. Some have a refrigerating outfit. Generally all have an abundance of steam for cleansing and sterilizing as well as for power.

Although these milk stations vary much in size and architecture, their external appearance has many common characteristics. Plate I shows one of the larger plants. On the left of the picture is shown the inclined roadway leading to the doors for receiving the milk and delivering the clean empty cans. The farmers' milk wagons reach these doors by the incline in order that the milk may be received at such an elevation that it can flow by gravity to the workrooms where it is handled. These plants are owned by large corporations with ample resources and represent a considerable investment. The owners have much capital and reputation at stake as an incentive to handling milk in a proper way. The plants are generally constructed and operated in a sanitary manner. They have thorough construction, with cement floors, tiled walls, enameled ceilings, and excellent plumbing, and are equipped with modern machinery. Plate II shows interior views of one of these plants. In one there is seen the process of bottling the milk and capping the bottles; in the other, the washing of the bottles.

One to four carloads of milk, cream, and buttermilk daily leave each of these bottling stations. They are owned by six concerns, and handle by estimation the product of from 4,000 to 4,500 producers. The number of towns from which milk is shipped in this way is approximately 50, located as shown in figure 2. McHenry County has about 20 plants. The two largest dealers have 23 establishments. Much of this milk is pasteurized in the country.

The milk cars containing this bottled milk are iced when necessary. They reach the city about midnight with the milk of that morning and the previous evening. They are switched at once to sidetracks controlled by the dealers and unloaded directly onto their platforms and transferred to the waiting wagons for the morning delivery. The two largest dealers have from 900 to 1,000 wagons each and each concern has 8 to 10 branches or divisions from which milk is distributed. The city "plants" of these dealers have almost no



GENERAL VIEW OF A HIGH-CLASS CHICAGO MILK-BOTTLING PLANT.

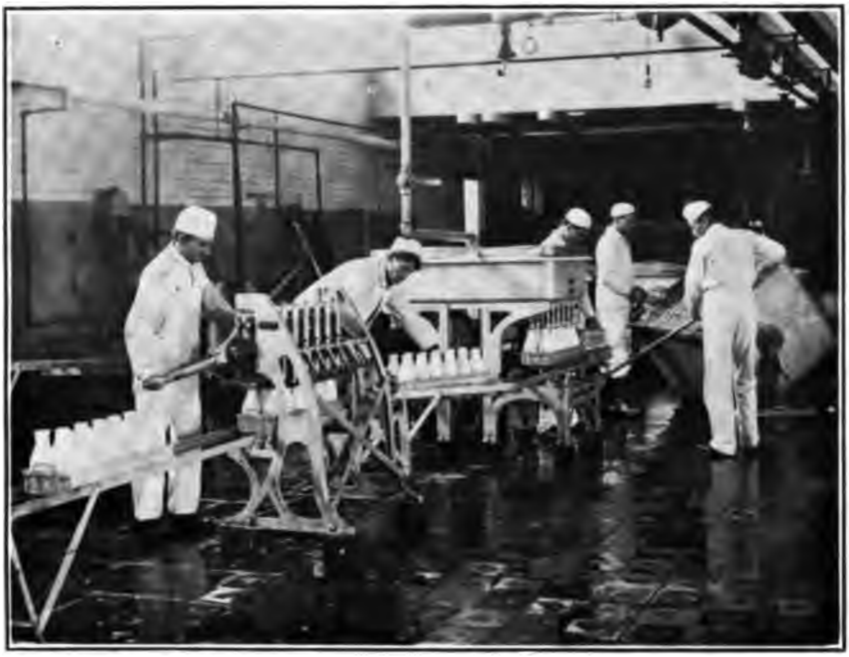


FIG. 1.—BOTTLING AND CAPPING MACHINES, AND TRACK ON WHICH BOTTLES TRAVEL IN THE CRATES. BOTTLE STERILIZER.

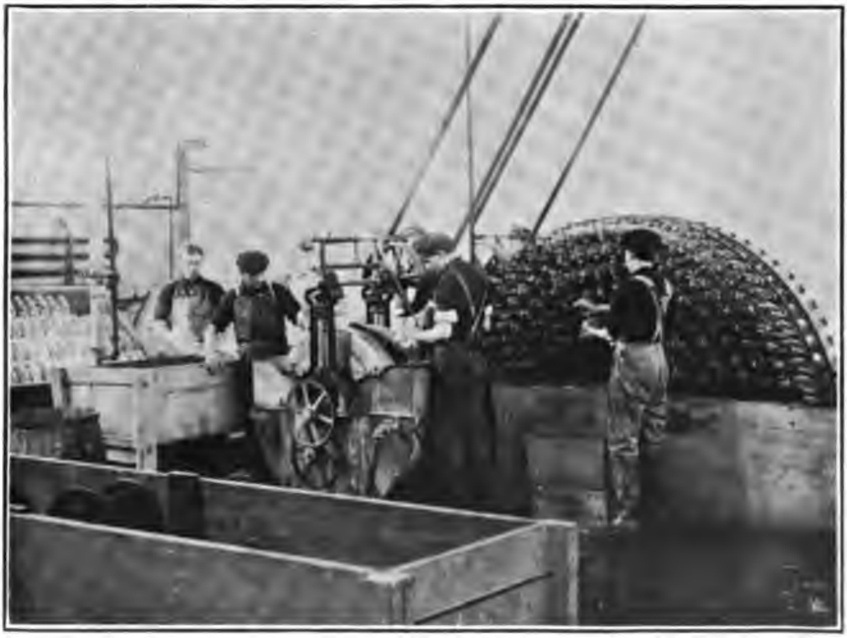


FIG. 2.—METHOD OF WASHING BOTTLES.

INTERIOR VIEWS OF PLANT SHOWN IN PLATE I.

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facilities for handling milk, and consist chiefly of a business office, stable, and laboratory. The large dealers order from day to day, by wire, from their country plants just what milk will be needed, as near as they can estimate. Hence, they never have more than a small, nominal surplus in the city, and need comparatively small city storage facilities.

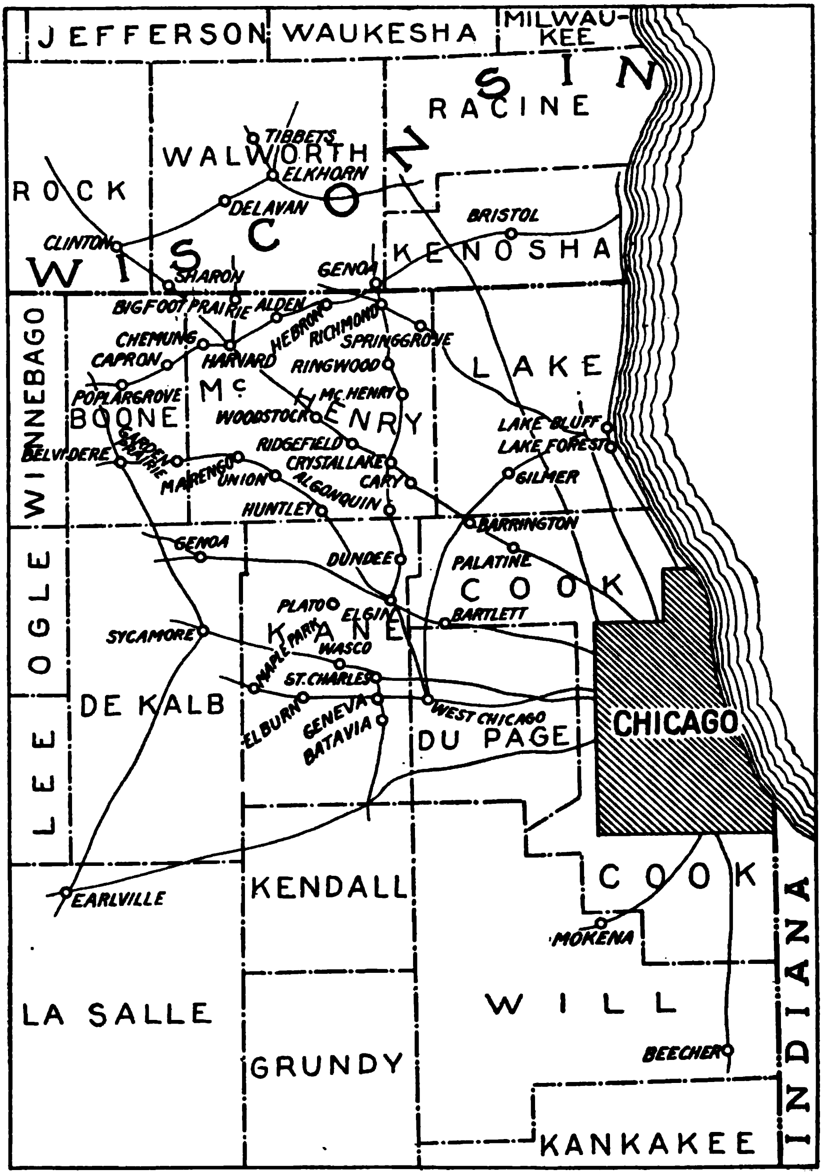

FIG. 2.—Map showing location of country milk-bottling plants supplying Chicago.

"Milk bottled in the country" has become locally a prominent watchword for advertising purposes. The expression is used freely on wagons, in booklets, and in the newspapers, with an implied assumption of superiority. This assumption in many cases has a foundation in fact if we compare the average bottling plant with some of the ordinary city plants where can-milk is indifferently handled. It does not necessarily follow, however, that milk bottled in the country is always in all cities superior to that bottled in the city. The persistence in pushing milk "bottled in the country" is one of the local peculiarities of the Chicago milk business.

METHODS OF THE SMALL DEALERS.

The small dealers receive their milk supply in the city at the railroad platforms, of which there are from 60 to 70, and haul it to their places of business. These dealers buy of the producers, who deliver the milk at the local stations, where it is collected by the milk trains. This milk is handled in 8-gallon cans.

The large dealers buy by the hundredweight, but the small dealers by the can. The producers for the small dealers are called shippers to distinguish them from the producers who sell in the country to the bottling plants; and the shippers' product is frequently spoken of as "can" milk. The small dealers have business places of every grade of quality and condition and with a wide range of equipment. At one extreme is the very small dealer with only a pushcart and a pouring can; at the other extreme is the thoroughly up-to-date establishment, with separator, churn, and pasteurizer, and other modern machinery. Though these small plants include a wide range of conditions there is a tendency toward general improvement. One of the leading men among the small shippers, stating the case from his viewpoint, said to the writer: "Some conditions are good and some are bad, but the average is constantly improving with the general progress being made and the steady weeding out of the worst places by the city health department."

Plate III shows one of the worst places (the exterior) and also one of the worst interiors. Plate IV represents interior and exterior views of one of the better plants.

Most of the milk handled by the small dealers is retailed in bottles; a 50-can dealer is considered one doing a relatively large business. The milk cans are transported on milk cars attached to passenger trains. These cars are usually common baggage cars, with steel floors and movable shelves which can be lowered to receive more cans than will stand on the floor. Sometimes on nearing the city the milk cars are dropped from the passenger trains and made up into special milk trains. The freight is paid by the shipper on the ticket system.

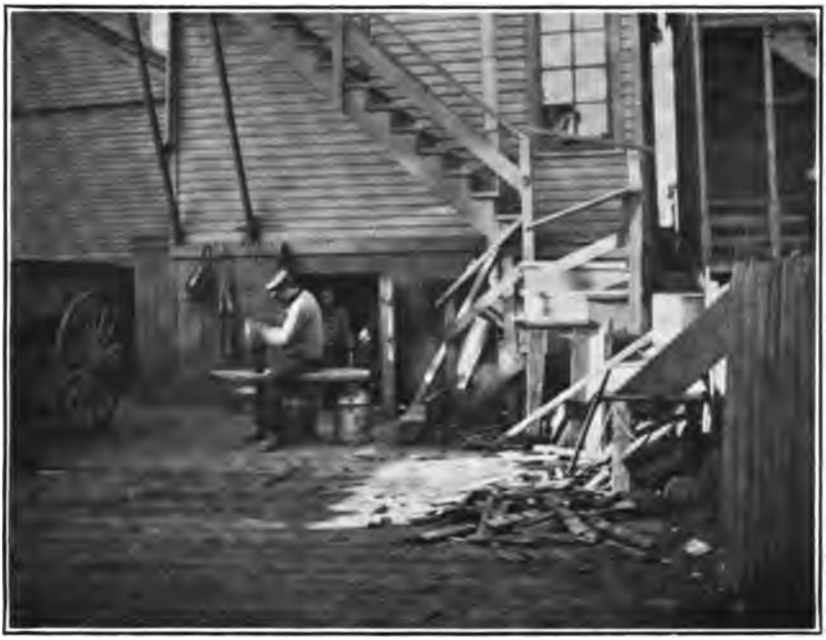

FIG. 1.—A REAR BASEMENT ESTABLISHMENT. DARK AND POORLY VENTILATED.

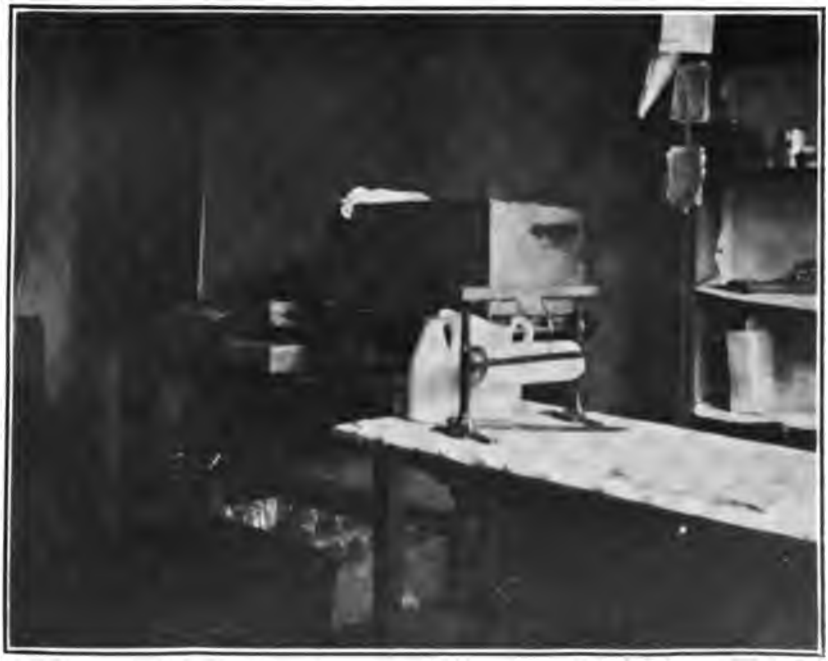

FIG. 2.—SMALL MILK PLANT IN FRONT BASEMENT ROOM. BOTTLING MACHINE NEAR END OF SHELVES. PITCHER FOR RETAILING MILK IS BESIDE WRAPPING-PAPER ROLL.

UNDESIRABLE CHICAGO MILK PLANTS.

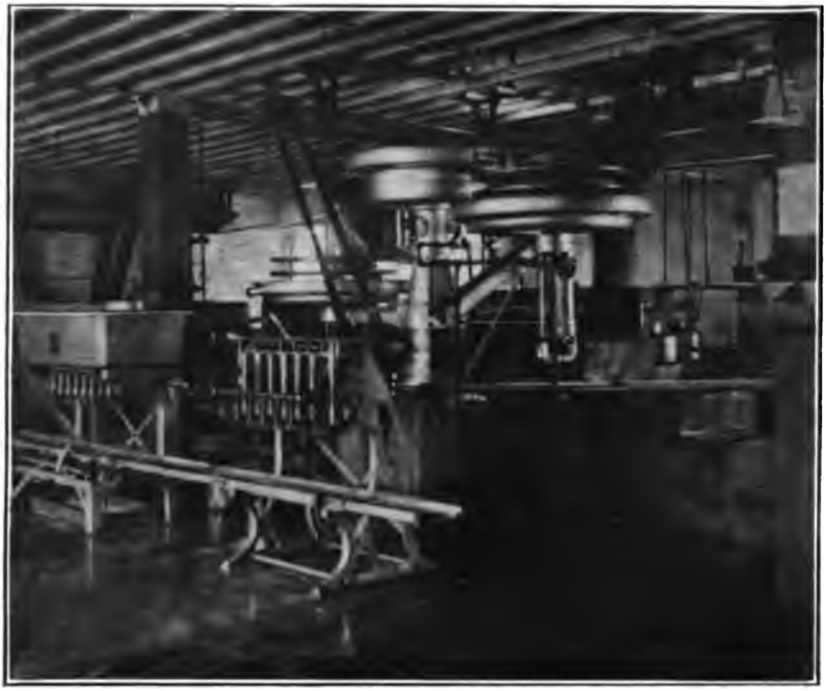

FIG. 1.—INTERIOR ARRANGEMENT, SHOWING PASTEURIZER, BOTTLER, AND CAPPER.

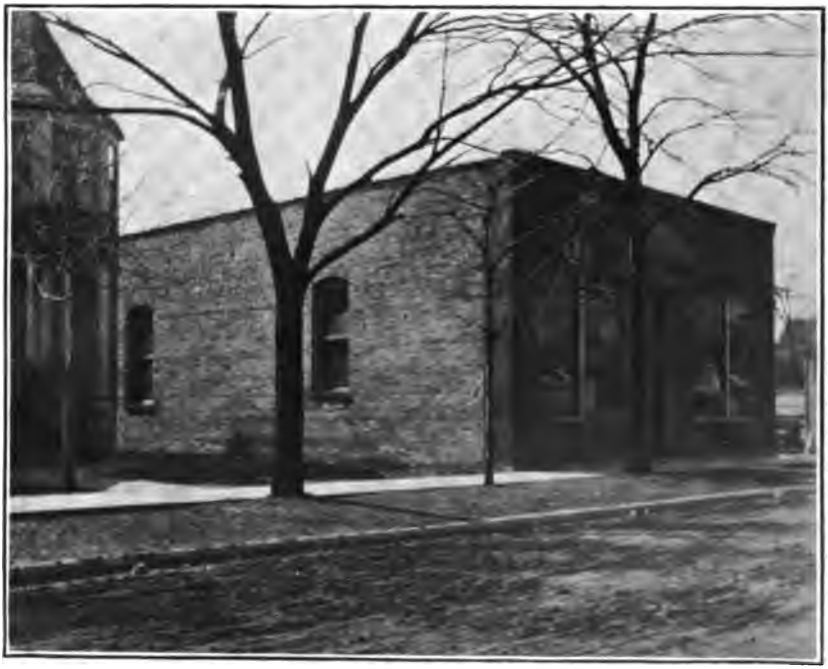

FIG. 2.—VIEW OF EXTERIOR.

CHICAGO CITY MILK PLANTS OF THE BETTER CLASS.

The number of the small dealers can not be determined from the city reports, which give only the number of licenses issued. This does not correspond with the number of dealers, for the health department issues licenses, or permits, to wagons rather than to individuals; consequently a single person may have a large number of licenses. Besides this, the number of dealers is constantly shifting, some dropping out and others coming in, so that the figures for one month would be inaccurate for another. However, by comparing various estimates and sources of information, the number of the smaller dealers is found to be approximately from 1,200 to 1,500, receiving the milk of from 5,000 to 5,500 producers.

The milk cars leave the country depots from 6 to 8 o'clock a. m. and arrive at 9 to 11 o'clock in the forenoon at platforms owned by the railroad company, some of which are covered and some are uncovered. The cars usually bring in the milk of that morning and the previous night. It is customary for the farmers to cool the milk somewhat at the farm, especially the night's milk, but nothing is done toward refrigeration in transit; therefore, as soon as the milk is received by the dealer, he at once thoroughly cools it. He then bottles it (usually in the afternoon) and stores it in ice water until time for the morning delivery. Farmers' shipments will range from one to a dozen cans each. Each shipper sends milk previously contracted for by some dealer, each can being tagged to the dealer—one tag stating the amount of the shipment. In this way the dealer readily gets at the platform the milk intended for him and can keep his accounts accurately with the producer.

There is a constant tendency among the small dealers to go out of business, saying, "There is nothing in it." This tends to concentration in the hands of the larger dealers.

HANDLING OF MILK IN STORES.

The experience of health officers in many cities has shown that grocery and provision stores and bakeries which sell milk are a menace. In the first place the proprietors of these stores frequently cut prices, sometimes selling milk at even less than the wholesale cost. These dealers use the low price of milk as an advertisement of their business, hoping to increase their sales of other articles by offering milk below cost; but such a course is demoralizing from the commercial side. In the second place, this store milk is often adulterated and is frequently handled in an extremely insanitary way—cans being uncovered in dirty places, dippers and measures extremely filthy, ice lacking, etc.

Boston has a bacteriological limit of 500,000 per cubic centimeter, and the relative percentages of the samples of milk that exceeded this number were found upon investigation by the local board of health to originate as follows:

	Per cent.
Place of production	1.5
Cars on arrival at city	12.4
Peddlers' wagons	45.6
Stores	71.5

Similar conditions existed in Chicago until recently. It was also ascertained that, as a rule, these stores were located in those districts of the city which had the highest infant mortality. Hence the common practice of dipping milk from the can in serving customers has been prohibited, and nothing but bottled milk is now allowed to be sold from stores. This regulation has been very beneficial in reducing the number of samples below grade in regard to the milk solids or food material.[1]

The number of stores in Chicago licensed to sell milk was 2,009 in 1908 as against 2,492 in 1907. In 1908, 6,732 store inspections were made and 810 stores were found insanitary; 784 of these were still bad on reinspection, and their licenses were revoked. This explains the falling off of about 500 in the number of stores selling milk in 1908. The number then increased under better conditions until it was 2,470 in 1909.

The ordinance requiring that all milk sold in stores should be bottled has resulted in great improvement in the purity of the article.

BROKERAGE.

A peculiarity of the Chicago milk business is the existence of a firm of milk brokers. This concern will undertake to find a customer for a producer who wishes to begin selling milk and will find a producer-seller for any middleman who wants to go into the business. In addition to acting in this way as a go-between for producer and dealer the brokers investigate credits and guarantee to shippers payment for their milk for a commission of 2 cents a can. This is at times important, for out of such a large number of dealers some will be dishonest and some though honest will be weak financially; hence there is an advantage in having some one to investigate the financial standing of dealers and guarantee accounts. Years ago the producers suffered heavy losses through the constant stream of dealers who failed to pay their bills. It is stated that never before in the history of the business has so little money been lost by the farmers by reason of bad credits as at the present time.

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[1] Report of Chicago Health Department, Feb. 20, 1909, p. 18.

ORGANIZATIONS.

PRODUCERS' ORGANIZATIONS.

The Milk Shippers' Union is an organization of the producers who ship milk in car lots to small dealers. Different localities have local unions who elect delegates to a central union. The union elects a board of directors, one from each line of railroad represented. The Milk Shippers' Union was incorporated under the laws of Illinois, November 13, 1897. Its objects are to improve the quality of milk; to prevent the loss and destruction of cans; to secure reasonable regulations from the railroads; to protect the milk shippers against dishonest dealers; to prevent controversies on price by having it understood before the milk is delivered; to prevent monopoly by keeping an open market for any individual shipper; to exchange information on the feed, the cattle, and the care of milk. Only the best dairymen are eligible as members. The union requires milk shipped to be pure, clean, sanitary, and to contain 3.6 per cent butter fat. It discusses and estimates the market value of such milk, based on the demand, the supply, the cost of production, and the wholesale prices paid by dealers in other large cities. It states its opinion as to what the price of milk should be; but in order to avoid any trouble under antitrust laws there is no contract, rule, understanding, or obligation as to a uniform price of milk among the members. Every producer sells his own milk at his own price, but the figure named by the union is generally the price asked by its members. Usually of late the price has been fixed on such a conservative basis that the dealers have paid it without question. The union has a membership of about 600 and has been productive of much good, especially when its income is considered. It has been run on from $78 to $200 a year in addition to a small salary for a secretary. The officers are: J. P. Mason, president, Elgin, Ill.; H. B. Farmer, secretary, 54 Randolph Street, Chicago, Ill.; S. B. Woods, treasurer, Crown Point, Ind.

The Illinois State Milk Producers' Institute holds an annual meeting for educational discussion. Prominent speakers are secured and interesting and profitable meetings are held. D. D. Hunt is president; J. M. MacVean, Chicago, is secretary.

In January and February, 1909, a movement was started to bring all the milk producers into one large, general organization. The organizers hoped that by uniting the interests of the producers who ship their milk in cans with those of the producers who sell to the bottling plants much greater power would be secured. The plan was helped on by some who felt that the shippers' union had not been radical enough, and by others who wanted united effort to combat the Chicago health rules. The plan of organization provides for a board of directors composed of one from each county having members in the association and an additional delegate from each of the five largest milk-producing counties. The directors are elected by the delegates from the local associations. The organization is called the Milk Producers' Association. The following are the officers: President, E. J. Fellows, St. Charles, Ill.; secretary, James P. Grier, Chicago, Ill.; treasurer, J. F. Martin, Antioch, Ill.

DEALERS' ORGANIZATIONS.

The 10 or 12 larger dealers have an organization called the Illinois Milk Dealers' Association.

The smaller dealers have an organization called the Chicago Milk Dealers' Association. This is subdivided into four local associations, one in each of the four main geographical sections of the city. At their meetings matters of mutual interest are discussed. Each local association elects four delegates to the central body. The latter considers the broader questions and employs an attorney and a business agent. The attorney looks after the interests of the members in courts, appears before committees of the city government to state the opinions of the milk trade on pending legislation, and does such other things as may be required from time to time. From one-half to three-fourths of the small dealers are in this association.

THE MILK DRIVERS' UNION.

The drivers of milk wagons are well organized, and this union has had much influence on the business as well as on wages. It has regulated the hours of work, by which all milk must be delivered before 1 o'clock p. m. in the summer. In the winter the drivers will not begin work before 8 o'clock a. m. Sunday hours of delivery are also regulated. A team will deliver from 200 to 300 quarts a day. There are about 1,700 members in the drivers' union.

WHOLESALE AND RETAIL PRICES.

The following tables give the wholesale prices paid by the milk dealers for a number of years. In the first table is given the can price as explained on page 16 delivered on the Chicago platform, the producer paying the freight out of this figure. The second table gives the price per 100 pounds paid at the large country plants by the bottlers:

Price paid per 8-gallon can for milk delivered in Chicago, 1896–1911.

Months.	1896	1897	1898	1899	1900	1901	1902	1903
January	$0.80	$0.90	$0.80	$1.00	$1.00	$1.10	$1.10	$1.15
February	.80	.90	.75	.80	1.00	1.10	1.10	1.15
March	.75	.75	.70	.80	.90	.95	1.00	1.10
April	.70	.70	.70	.80	.90	.90	.95	1.05
May	.60	.65	.65	.65	.75	.75	.75	.80
June	.60	.65	.65	.65	.75	.75	.75	.80
July	.65	.75	.70	.75	.85	.85	.85	.90
August	.65	.75	.75	.85	.85	.90	.90	1.00
September	.70	.75	.85	.90	.95	.95	.95	1.00
October	.70	.80	.85	.90	.95	.95	.95	1.00
November	.85	.85	1.00	1.15	1.15	1.15	1.15	1.15
December	.87	.80	1.00	1.10	1.15	1.15	1.15	1.15

Months.	1904	1905	1906	1907	1908	1909	1910	1911
January	$1.15	$1.15	$1.10	$1.20	$1.45	$1.30	$1.40	$1.55
February	1.15	1.10	1.10	1.15	1.45	1.30	1.35	1.50
March	1.15	1.10	1.05	1.10	1.30	1.25	1.30	1.45
April	1.05	1.00	1.05	1.10	1.25	1.25	1.30	1.40
May	.75	.80	.80	.90	.95	1.00	1.00	1.40
June	.75	.80	.80	.85	.90	.90	1.00	.95
July	.85	.85	.90	1.00	1.00	1.00	1.10	1.05
August	.85	.85	.90	1.05	1.10	1.10	1.15	1.20
September	.95	.95	1.00	1.10	1.10	1.15	1.25	1.25
October	.95	.95	1.00	1.10	1.15	1.15	1.25	1.25
November	1.15	1.15	1.20	1.45	1.35	1.45	1.60	....
December	1.15	1.15	1.20	1.45	1.35	1.45	1.60	....

Price paid per 100 pounds for milk delivered at bottling establishments in the country.

Months.	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911
January	$1.35	$1.45	$1.45	$1.55	$1.50	$1.45	$1.55	$1.65	$1.55	$1.75	$2.00
February	1.35	1.40	1.45	1.50	1.40	1.40	1.45	1.65	1.55	1.70	1.90
March	1.30	1.30	1.35	1.40	1.30	1.30	1.35	1.55	1.45	1.55	1.70
April	1.05	1.15	1.20	1.20	1.20	1.20	1.30	1.35	1.40	1.45	1.30
May	.85	.90	.95	.90	.90	.90	1.00	1.05	1.10	1.20	1.10
June	.85	.80	.85	.80	.80	.80	.90	.95	.95	1.05	1.10
July	.80	.90	.95	.90	.90	.90	1.00	1.05	1.05	1.20	1.30
August	1.05	1.05	1.10	1.10	1.05	1.10	1.20	1.25	1.25	1.30	1.45
September	1.15	1.15	1.20	1.20	1.15	1.20	1.30	1.35	1.35	1.40	1.45
October	1.30	1.35	1.35	1.30	1.30	1.35	1.55	1.45	1.55	1.70	......
November	1.40	1.45	1.45	1.40	1.40	1.45	1.65	1.55	1.70	1.90	......
December	1.45	1.45	1.55	1.50	1.45	1.55	1.65	1.55	1.75	2.00	......

The bottlers contract for milk for six-months' periods in April and October; the shippers fix their prices in May and November.

Figure 3 gives a graphic view of the prices paid beginning with 1903 by the two systems, reduced to the quart as a unit. The upper line represents the shippers' price and the lower line the bottling-plant price. In the former case the farmer pays the freight, which averages half a cent a quart (see p. 12). The dotted line shows the net return to the farmer who ships can milk to Chicago. Figure 4 shows the maximum and minimum prices for each year since 1896.

The prevailing retail price in the autumn of 1907 was 7 cents a quart, and had been for some time. An attempt to raise the price to 8 cents was made in the winter of 1907–8. This was followed by

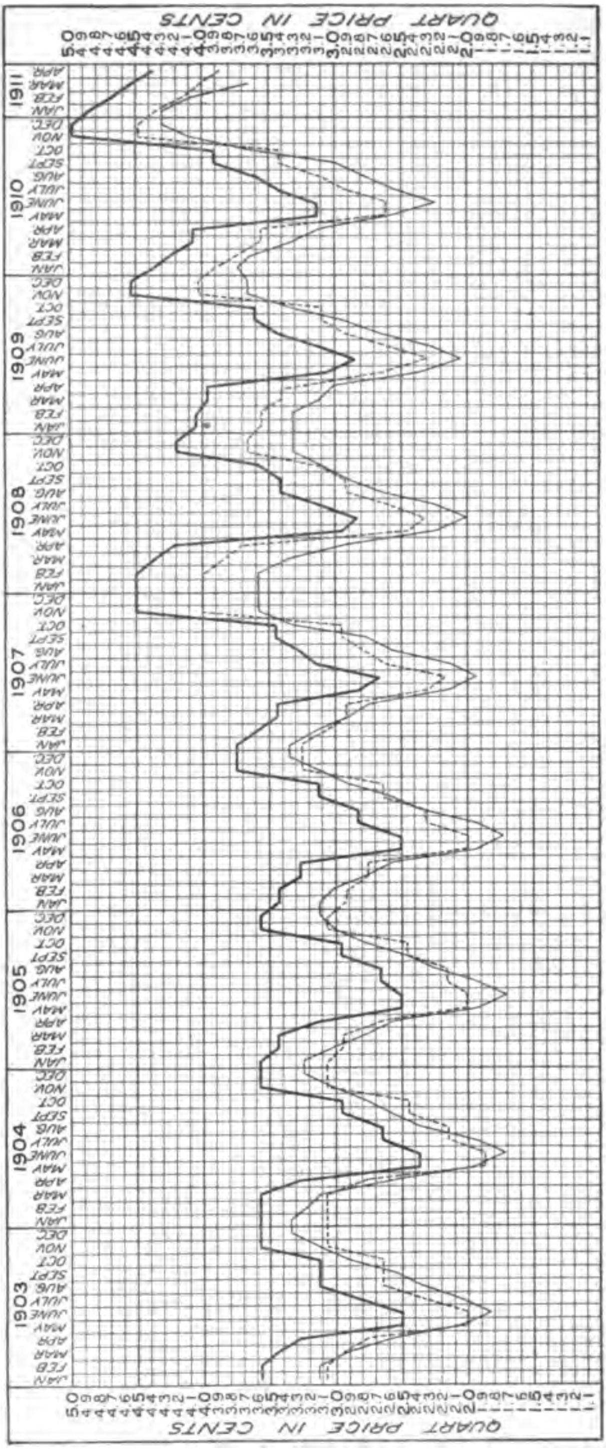

FIG. 3.—Monthly range of wholesale milk prices at Chicago.

cries of "trust" in some of the newspapers and 13 grand-jury indictments. It was charged—

> That defendants were engaged in a conspiracy to crush out competition; that defendants conspired to form a pool; that defendants conspired to commit an illegal act against the public administration of justice; that the conspiracy was not only to control prices charged to consumers, but to regulate prices paid to producers.

The bill of particulars filed by the Commonwealth alleged—

> That defendants advanced the price of milk to consumers 1 cent per quart in the fall of 1907 almost simultaneously.

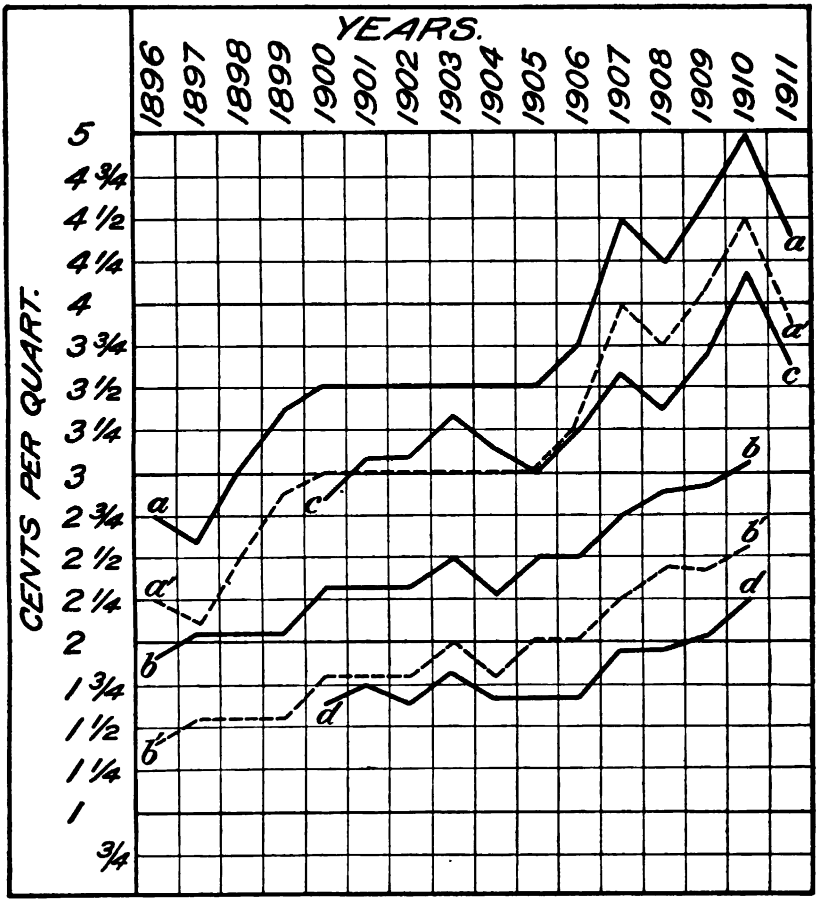

FIG. 4.—Summer and winter wholesale milk prices at Chicago. *a*, Price of can milk in November and December; *a'*, same less freight; *b*, price of can milk in May and June; *b'*, same less freight; *c*, winter price of milk at country bottling plants; *d*, summer price at bottling plants.

> That customers received notice from defendants about the same time regarding the increase in milk prices.
>
> That defendants increased the price given to farmers in order to drive out competition in the dairy-produce country.

As a result of this agitation the retail price was dropped to 7 cents and remained at that price during the winter of 1908–9. When the case was reached on the court calendar it was dismissed by the judge without trial.

In the winter of 1909–10 an effort was again made to raise the price to 8 cents, but much milk was sold at 7 cents, and daily newspaper agitation finally reduced the price to 7 cents.

THE OFFICIAL INSPECTION.

Milk inspection in Chicago is under the general supervision and direction of the health commissioner and is directly in the division of food inspection. This division has a chief, and also a chief of dairy inspection. Under the latter there are at present 10 milk inspectors for city work and 10 dairy inspectors for country service. The salaries of these 21 officers aggregate $22,500. The health department has a laboratory with 3 bacteriologists and 6 chemists for general work, and in addition 2 officers specially designated as milk testers. These latter are paid $2,400, increasing the direct expense for milk inspection to $24,900, no account being taken of other office and supervisory expenses, which might properly be chargeable to milk inspection.

The milk ordinance now in force was adopted early in 1908, and the score-card system of inspection was soon after inaugurated. There are used one form of score card for dairy farms, substantially like that of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture, another form for city milk depots, and a third for stores selling milk. The health department has also devised a form of score card for the inspection of bottling plants.

In addition to the official inspection, the largest bottlers have regulations looking to clean, pure milk, and employ their own inspectors to see that these regulations are complied with.

A question has been raised, growing out of the increased activity of the health office, as to the legal rights of city inspectors when making investigations outside of the municipal limits, an eminent authority having said that these inspectors—

> have no more authority upon your farm or mine than the burglar that seeks to break into our house at night.

On the other hand, the ordinance says that—

> Milk produced on farms * * * where the rules of the department are violated shall be declared unclean, unwholesome, and infected. The sale of or offering for sale of such milk is prohibited. All unclean, unwholesome, or infected milk shall be condemned for human food.

Such milk is to be returned to the shipper, and on a second offense is to be so denatured that it can not be used as food. Hence, without raising technical or academic points, it will be seen that the producer will save himself annoyance and inconvenience by allowing an inspection of his premises.

The milk laws in effect in the city are for the most part municipal ordinances. Where the health officer or other interested party desires a new law or the modification of an old one, he goes to the city council rather than to the legislature. The present Chicago ordinances are very specific as to all details of production and handling. The sale of milk over 70° F. or containing over 3,000,000 bacteria per cubic centimeter is prohibited.

In July, 1908, the city council passed an ordinance which provided that all milk sold in the city should be from cows that had satisfactorily passed the tuberculin test; but, realizing the difficulty of putting such an ordinance into immediate effect, the ordinance allowed pasteurization as an alternative for five years. The theory upon which the ordinance was passed was that five years would be required to test the cows and exclude the tuberculous, there being about 125,000 cows producing milk for the Chicago market, owned by some 12,000 farmers. When the ordinance was passed it was to take effect January 1, 1909, but bitter opposition developed and it has been fought with persistency for several years. The time for its going into effect has been postponed from time to time, until finally it became effective January 1, 1911. After that date no license has been issued to any dealer unless his supply was properly pasteurized or obtained from a dairy the cows of which had been properly tuberculin tested within one year.

Growing out of this agitation, a city commission was appointed to investigate conditions and a State commission was appointed to make an investigation as to the accuracy of the tuberculin test. The first commission has made a preliminary report critical of the city milk supply, and the State commission has reported to the legislature a recommendation to the effect that municipalities should not have the right to enact tuberculin testing ordinances.

Meanwhile the work of testing cows progressed. Animals were tested with tuberculin as follows: In 1908, 10,860; 1909, 29,499; 1910, 6,302. But the recent session of the legislature enacted the following: "It shall be unlawful for any city, village, incorporated town, county, or other corporate authority in the State of Illinois by ordinance, rule, or regulation other than may be established by the law of this State to demand, fix, establish, or require the tuberculin test to be applied to dairy animals as a means or measure of regulating and purifying milk, skimmed milk, cream, and dairy products of said animals in any manner whatever, and every such ordinance, rule, by-law, or regulation heretofore or hereafter passed, demanded, fixed, established, or required by any such city, village, incorporated town, county, or other corporate authority other than the State of Illinois is hereby declared to be void and of no effect."

The number of pasteurizers installed in the city is 210 and in the country 100—310 in all. Most of these have been put in during the past two years.

The inspection of the work of pasteurization devolves upon the health department. The number of tests made in 1909 was 3,914; in 1910, 5,203. The tests show good results from the standpoint of reduction of the bacteria, but are nevertheless unsatisfactory and show much poor work done by the pasteurizers, which the department is laboring to overcome. The average bacteria count from August 1 to December 31, 1909, was 944,000 per cubic centimeter, and 2,219 of the 1910 samples averaged 1,702,000. The average of the city's raw milk for 1910 was 11,574,000 and during the summer months it was 13,932,000.

During the seven weeks ending the middle of September, 1909, the inspectors of the health department took 829 samples of milk for bacteriological examination, with the following results:

Results of bacteriological examination of 829 samples of milk in Chicago.

Bacteria per cubic centimeter.	Pasteurized.		Raw.	
	Number.	Per cent.	Number.	Per cent.
Less than 1,000	2	0.76	....	....
1,000 to 10,000	8	3.04	....	....
10,000 to 100,000	94	35.90	14	2.47
100,000 to 500,000	80	30.52	57	10.04
500.000 to 1,000,000	31	11.80	45	7.93
1,000,000 to 5,000,000	34	13.00	227	40.03
5,000,000 to 10,000,000	5	2.00	106	18.70
10,000,000 to 20,000,000	7	2.60	81	14.30
Over 20,000,000	1	.38	37	6.53
Total	262	100.00	567	100.00

All of the samples of unpasteurized milk averaged 5,547,502 bacteria per cubic centimeter; the pasteurized milk averaged 944,465.

One section of the ordinance requires that drivers' seats on milk wagons shall be divided from the compartment where the milk and cream are kept. Milk for delivery in bulk must be carried in covered pouring cans provided with a spout or faucet. It must not be dipped from carrier cans for delivery to consumers. All milk shipped into Chicago must be sealed. The last rule, in the estimation of the health officer, has almost entirely stopped the watering of milk. Sealing the cans has a tendency to fix the responsibility for adulteration, and therefore makes conviction and punishment more certain, which has had a decidedly deterrent influence.

An average of 1,230 samples are taken each week. It is expected that, as a rule, a dairy-farm inspector will make 40 inspections per week, and that the city inspector will make 30 inspections with the necessary reinspections and take 100 samples. The following shows in detail the work of inspection:

	1907	1908	1909	1910
Samples of milk taken in the city for chemical analysis	60,688	63,984	41,853	34,739
Per cent below grade	7.4	4.8	4.2	3.5
Cases in court	1,473	2,689	1,312	1,201
Fines imposed	$667	$7,880	$1,305	$920
City inspections of stores and milk depots	6,638	16,855	16,566	15,808
Cases in court	47	352	1,442	925
Fines imposed	$282	$2,112	$5,846	$4,756
Inspections of dairy farms	7,374	9,865	10,211	5,221
Farms whose supply was excluded from the city	468	350	....	262

The falling off in samples taken for chemical analysis is due to the increasing attention given to bacteriological tests and is offset by the increased number of such examinations. Increasing attention has been given during the past few months to filtering samples of a pint each through a small disk of cotton. These are preserved with the name of the dairy from which they were obtained and, if bad, used as a lever to secure better conditions.

EXISTING CONDITIONS.

In discussing the quality of an inspection system which has to oversee 12,000 milk producers and 1,500 city milk dealers with 2,600 wagons and 2,400 stores, with only 20 inspectors, much will depend on the viewpoint and temperament of the critic. He will find much to commend and much to criticize, and criticism of conditions may not imply criticism of the inspection service or the faithfulness and efficiency of its work. Such a tremendous job can not be perfect in results, and there will necessarily be various grades of efficiency where many inspectors are employed.

As a result of some investigations made in 1905–6 by Prof. J. M. Trueman, of the Illinois Agricultural Experiment Station,[1] he reported that—

> The absence of preservatives was plainly noticeable in Chicago milk; that of 413 samples of milk taken in the summer of 1906, 134, or 32 per cent, were below the standard (3 per cent of fat); 21 of these, however, had 2.8 and 2.9 per cent of fat, and were so near the line as not to be cases for prosecution. It was an exception to find a milk depot that was clean and sanitary. A great many of the markets are located in dark, dirty, and ill-ventilated basements where the sunlight never enters. They are never scrubbed, and many of them could not be because the floors are rotten wood or only of earth. Occasionally one of these small depots is found scrupulously clean.

Conditions have improved since that time, as has been shown on previous pages, with a new law, new energy, and more inspectors; and evidence is abundant that steady progress is being made, though much remains to be done, and Dr. W. A. Evans, health commissioner, himself said, in March, 1910: "Chicago's milk supply is one of the dirtiest in the world."

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[1] Bulletin 120, Illinois Agricultural Experiment Station.

The writer of this bulletin spent a part of November and December, 1909, in some investigations, working a few days with each of the inspectors and seeing a little of each milk-producing section. In every place visited the Chicago inspector and the writer visited every dairy they came to so far as the time would allow, so that there was no selecting of dairies, and each inspector asserted that a fair average of the conditions was seen. Speaking in a general way, conditions were bad, but everywhere there were evidences of improvement. Stating the results of the investigations in mathematical terms, resulting from the rating given on the score cards, 100 being perfect, there were found:

In the twenties, 11.7 per cent of the places visited.
In the thirties, 35 per cent of the places visited.
In the forties, 43 per cent of the places visited.
In the fifties, 9 per cent of the places visited.
In the sixties, 1.3 per cent of the places visited.

The average was 40.06.

Seventy-three per cent were rated nothing for cleanliness of the stable yard; 59 per cent were given a score of nothing for light; and a score of nothing for ventilation was given to 42 per cent.

In the majority of cases cows were allowed to calve in the stanchions. The milk room in most of the dairies was simply a tank room where the milk is cooled. In many cases this was badly located, being at the corner of the barnyard or a hog yard or both. In practically every case the milk utensils were washed in the kitchen of the house. No cooler was found in any instance, milk being cooled by being set in a tank of well water. Not a single narrow-top milk pail was found in use, or a single instance where milking suits were used. Eighteen per cent of the cows were given a score of nothing for cleanliness. Forty-six per cent of the dairies inspected were scored nothing for cleanliness of the stable air at the time of milking. A score of nothing for cleanliness of milk house was given in 41 per cent of the places visited. Eleven per cent of the dairies received a score of nothing for care and cleanliness of utensils.

The external appearance of the barns in the milk-producing territory is much better than in some other sections of the United States. A large proportion of them are in good repair and well ventilated, but many appear to be deficient in light.

The department of health a few years ago waged a vigorous contest against the feeding of wet malt to dairy cows. Powerful interests attacked this ordinance, but the appellate court of Cook County affirmed the validity of the ordinance October 8, 1906. The court said that power to enact ordinances for the preservation of the public health is inherent in a municipality, and quoted an opinion of the Supreme Court of Minnesota (66 Minn., 166), which said that—

> Any public regulations that did not provide means for insuring the wholesomeness of milk * * * for sale and consumption would furnish very inadequate protection to the lives and health of its citizens. * * * The presumptions of the law are in favor of the reasonableness of the ordinance. * * * It is therefore incumbent upon anyone who seeks to have it set aside as unreasonable to point out or show affirmatively wherein such unreasonableness consists.

INFANT MORTALITY.

The health commissioner reported in February, 1909:

> The infant death rate of the last few weeks shows a material improvement. This may be due to an improvement in general sociologic conditions. Possibly life is not quite so hard as it was a year ago. Nothing responds so accurately to the financial conditions of the poorer people as does infant mortality. And possibly also all of this agitation for a better milk supply is showing in an improved baby death rate, as it has been shown in the statistics of chemical and bacteriological examinations of the milk division.

In his annual report for 1909, published in January, 1910, Commissioner Evans said:

> A most noteworthy development of the year has been the pronounced reductions in deaths among milk-fed babies—in spite of an unusually hot summer. It is believed that a purer milk supply has contributed most in this reduction. With 60 per cent of the city's supply now pasteurized, and with regulations for the sanitary handling of milk more universally enforced than ever before, our babies' milk has, within the last year, become a much safer and better article of food. Under ordinary conditions it is a certainty that greater reductions would have been recorded. As it stands there were 641 fewer baby deaths as compared with 1908, a reduction that is not only gratifying but is regarded as hopeful for much better things in the future.

The following table shows the decrease in infant mortality between the ages of 1 to 5 years for four decades:

Decade.	To 1,000 population.	Per cent of total mortality.
1871–1880	4.69	22.84
1881–1890	3.85	18.67
1891–1900	2.60	14.20
1901–1910	1.42	9.69

THE PRODUCTION OF HIGH-GRADE MILK.

Milk of high grade is produced by a number of dairies and sold at several cents a quart above the price of ordinary milk. A few dairies have sold for several years what they called certified milk, but the term in its strict sense has been a misnomer. The expression "certified milk" is correctly used only when the milk has been produced under conditions prescribed by a committee of some regular medical association and when this committee certifies that its rules or recommendations have been complied with.[1]

Usage has given the name of "commission" to these committees. The earliest commission of a medical society in the field was the Essex County Milk Commission of Newark, N. J., which copyrighted the term "certified milk," but offered it for use by any medical milk commission organized to influence dairy work for clinical purposes. The use of the word "commission" as applied to the milk committee of a medical society is liable at times to give rise to confusion, as was the case in Chicago for some time. This city had no milk commission, in the sense mentioned above, until recently, and hence no body which could certify milk, though large amounts of so-called certified milk were sold. To increase the confusion, an organization adopted the name of "milk commission," to "distribute pasteurized and modified milk among sick children of the poorer parts of Chicago at prices far below cost." This philanthropic body did not exist for the purposes of certifying to the satisfactory production of milk, and only a minority of its officers were physicians. However, it did most excellent work in its way. For instance, in January, 1909, it distributed 59,360 bottles of milk from its own laboratory and from 21 other stations. This was an increase of 12,545 bottles over the amount distributed during the corresponding month of the previous year.

A regular medical milk commission for certifying milk was appointed by the Chicago Medical Society early in 1909 and is now certifying to the milk produced by seven dairies. The certified milk is sold for the most part by regular dealers, who also handle the common market milk. Dr. J. W. Van Derslice is president and Dr. Julia D. Merrill is secretary of this commission.

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[1] Certified milk in the strict sense of the term is milk produced under a legal contract between a medical milk commission and a dairy and which conforms to its requirements.—C. B. Lane, U. S. Dept. of Agriculture, Bureau of Animal Industry Bulletin 104, p. 9.

"Certified milk" is a term * * * coined by the writer and first used in connection with a plan brought to the attention of physicians * * * in 1893. * * * It provided for a commission of medical men who should by voluntary supervision, paid expert inspection, and final certification, endeavor to influence a supply of milk produced under regulations imposed by themselves.—Henry L. Coit, M. D., Kentucky State Medical Journal, May, 1908.

No person shall sell or exchange or offer or expose for sale or exchange as and for certified milk any milk which does not conform to the regulations prescribed by and bear the certification of a milk commission appointed by a county medical society, organized under and chartered by the Medical Society of the State of New York, and which has not been pronounced by such authority to be free from antiseptics, added preservatives, and pathogenic bacteria or bacteria in excessive numbers.—New York Laws, sec. 22, amended April 30, 1907.

Part II.—THE MILK SUPPLY OF WASHINGTON.

AMOUNT AND SOURCE OF THE SUPPLY.

The District of Columbia consumes about 76,000 quarts (19,000 gallons) of milk a day, or about 0.4 of a pint per capita. This is produced on 1,091 dairy farms, from 17,688 cows. A few years ago when the number was 16,272, they were located as follows:

	Cows.
District of Columbia[1]	734
Maryland	8,448
Virginia	6,428
Pennsylvania	137
New York	525
Total	16,272

The District is surrounded on all sides by good farming country, and if dairying were intensively developed the entire milk supply could be easily produced within wagon haul of the District. As it is, about one-third is brought in by wagons and two-thirds by steam and electric railroads. Of the milk brought into the District by the latter conveyances 59 per cent comes by the Baltimore & Ohio Railroad, 30 per cent by the Southern lines, and 11 per cent by electric lines. Most of the supply is produced within 55 miles of the city. The bulk of it comes from Loudoun and Fairfax Counties, Va., and Montgomery and Frederick Counties, Md. (See fig. 5.)

The near-by supply of cream is insufficient, and considerable quantities are received regularly from Philadelphia and New York. The dealer can, in an unforeseen emergency, telegraph to some city depot or country creamery at these places and receive a supply within a few hours.

TRANSPORTATION AND FREIGHT.

The Baltimore & Ohio Railroad delivers from 6,000 to 6,500 gallons daily. It has about 20 milk stations, mostly on the Metropolitan Branch, the most distant shipping point being 57 miles away. The largest amounts are shipped from Germantown, Boyds, and Frederick, all in Maryland.

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[1] The total number of cows in the District, including the cows kept by public institutions and by individuals owning only one cow for family use, is 1,762.

The Southern Railway transports about 3,300 gallons daily. This road has 38 shipping stations on the main line and two branches, the most remote point of taking milk being 92 miles distant from the city. The largest shipping points in its territory are Herndon and Purcellville, in Virginia.

The milk is brought to the stations by the farmers in their own cans and is shipped in baggage cars attached to the passenger trains. Plate V, figure 1, shows a common morning scene at one of these railroad stations. The farmers have unloaded the milk from their wagons to the platform before the arrival of the train, and while the train waits they rush the cans into the car. This picture is, in fact, characteristic of market-milk production in many other parts of the country, similar conditions and scenes being found in every State where milk is produced for and shipped to a city market.

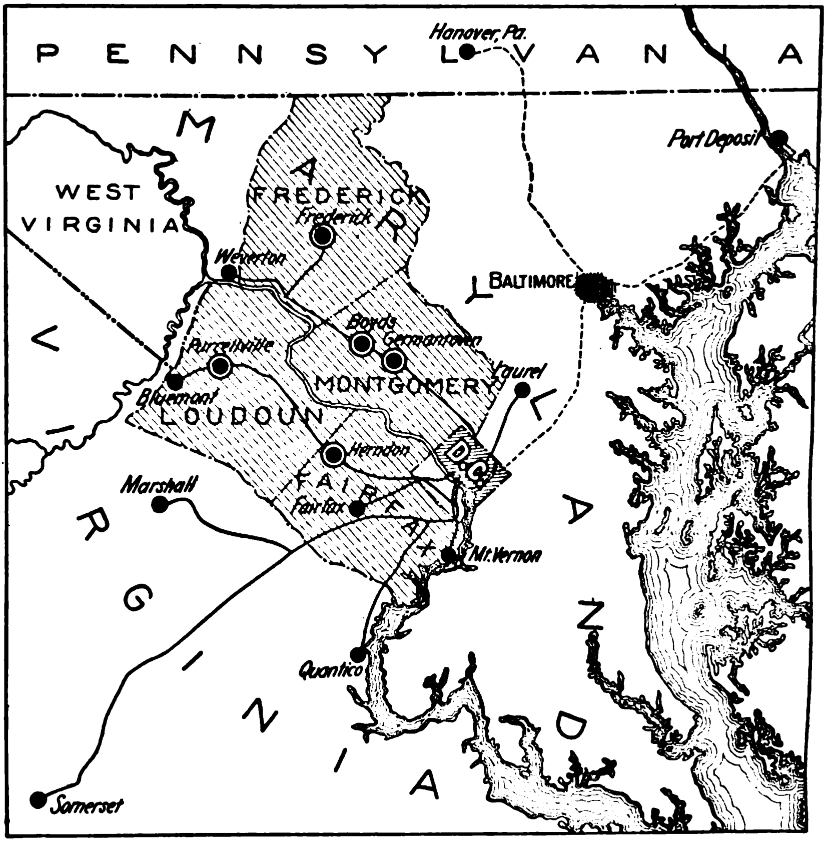

FIG. 5.—Map showing source of most of Washington's milk supply. Heavy dots indicate points from which milk cars start. Five largest shipping points are shown by dots inclosed by circles.

In the construction of the new Union Station at Washington provision was made for the convenient and prompt handling of milk by a siding and platform 600 feet long at the rear of the station, to

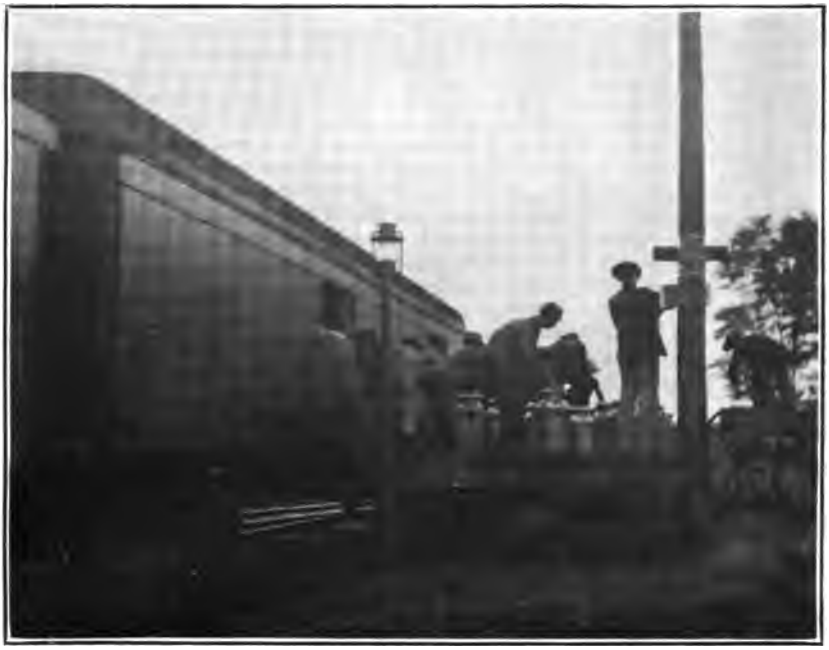

FIG. 1.—LOADING A MILK CAR BEFORE SUNRISE IN VIRGINIA.

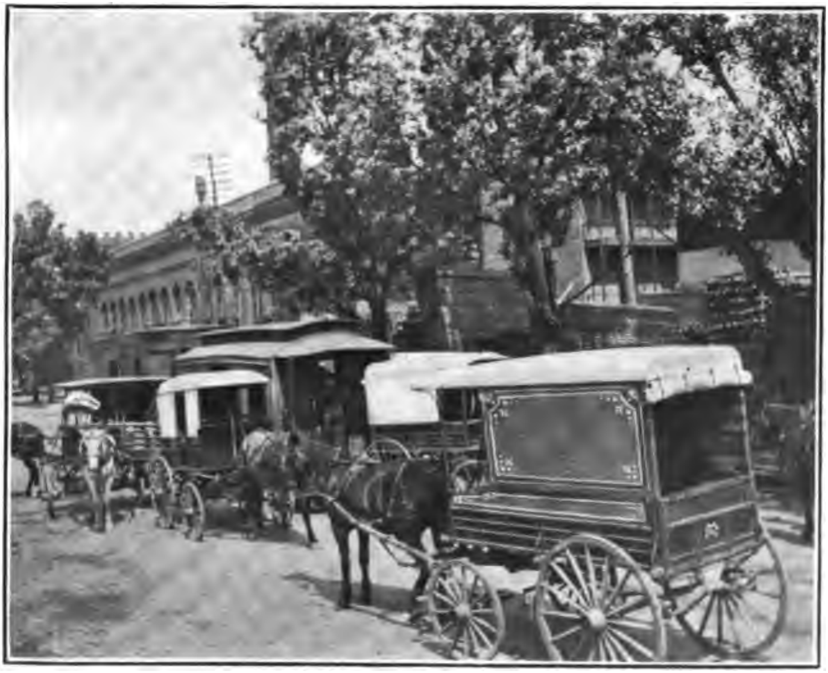

FIG. 2.—UNLOADING MILK FROM AN ELECTRIC CAR IN A WASHINGTON STREET.

HANDLING MILK FOR WASHINGTON.

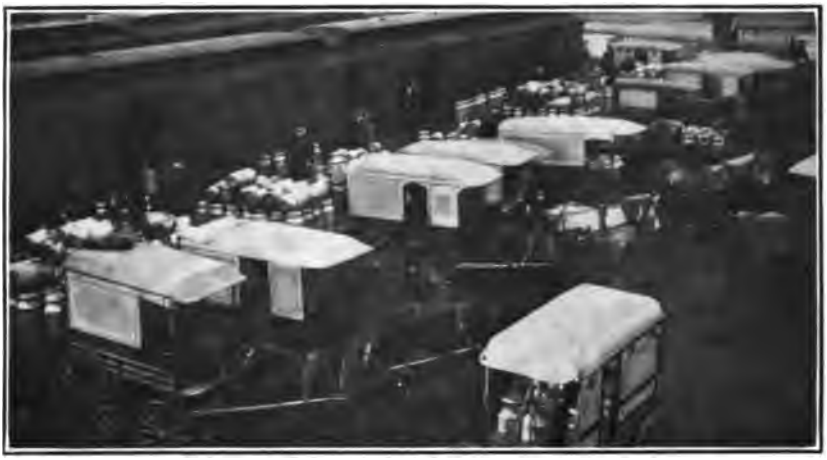

FIG. 1.—SCENE AT MILK PLATFORM AT OLD BALTIMORE & OHIO STATION.

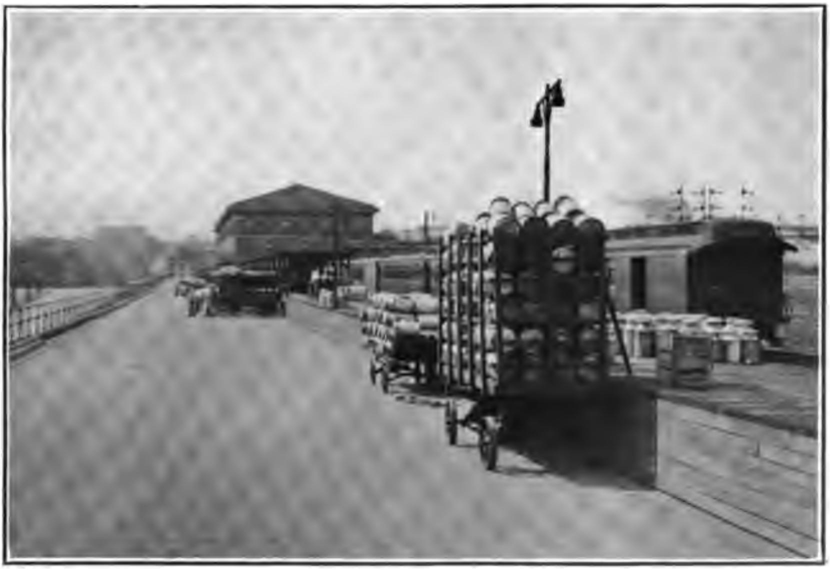

FIG. 2.—MILK PLATFORM AT NEW UNION STATION.

UNLOADING MILK AT RAILROAD STATIONS IN WASHINGTON.

which the cars can be quickly shifted on arrival. (Pl. VI, fig. 2.) The cars are for the most part due to arrive between 8 and 9.30 o'clock a. m., although, on account of the lack of refrigeration, afternoon shipments are sometimes made in summer.

Electric railroads are utilized for milk shipments into Washington more than in many places of this size, and one road brings in milk by the carload. (Pl. V, fig. 2.)

FREIGHT RATES.

Most of the milk shipments are in 40-quart cans, though some twenties and thirties are used. The freight rate on the steam roads is from 2 to 3 cents a gallon, according to distance. Most of the milk comes from the 2½-cent zone, making the average freight a little over half a cent (0.6 cent) a quart. On the electric roads the rate ranges from one-half cent to 1½ cents a gallon. The custom prevails of making the same charge for cream as for milk. Freight is paid by tickets attached to the cans.

THE PRODUCERS.

The 1,091 farmers who produce milk for the District of Columbia have herds averaging about 16 cows in each. Only 32 exceed 50 cows, and only 4 farmers have herds exceeding 100 cows each. On the other hand, 230 farmers have 10 cows or less. The great majority of the herds number from 10 to 30 cows each. The tendency, however, is to increase the size of the herds. The statistics show that there is a decreasing number of producers, while the growing needs of the city call for the product from an increasing number of cows.

Conditions attending the production of milk are improving. The District health department, although hampered by limitations which will be referred to later, has done commendable work in eliminating some of the worst features connected with the production of the local milk supply. In 1906–7, 241 cows were condemned as unfit for milk production, and in 1908–9, 220 were condemned. The farms supplying the milk were scored by the Dairy Division in 1906–7, the average score being 43.44, and 300 scored in the thirties or below, the latter scores indicating conditions which would warrant excluding the product of such dairies from the market. Ventilation was the condition which scored lowest; next to the lowest was removal of manure; and next, light. Other noticeably bad conditions related to cooling the milk, cleanliness of milking, cleanliness of stable yard, and construction of stable. Marked improvement has been made since then, but Dr. W. C. Woodward, the District health officer, says:

> The improvement seems slow. The public, however, which has been for many years so tolerant of insanitary conditions on the dairy farm, must realize that existing

conditions are due to its own ignorance and neglect quite as much as to the ignorance and neglect of the farmer.

Sixty-two dairymen, keeping 734 cows, reside within the District limits and retail their product without the intervention of middlemen.[1] Milk is also brought into the District by wagons from about 25 villages, 18 miles being the longest haul. Much of this wagon milk is retailed by the producer, though a considerable proportion of it is taken to the city milk plants and handled the same as railroad milk.

METHODS OF HANDLING MILK IN THE CITY.

The District of Columbia has about 79 middlemen who have regular milk plants. This is a larger number than is usual in places of this size. By a localism these dealers are called "dairymen" and their plants are called "dairies." The latter include a wide range of conditions, from the establishment in the rear basement of some dwelling with meager light and air to the up-to-date place with a full equipment of modern machinery. (See Pls. VII and VIII.) The dealers do a business ranging from 50 to 2,000 gallons a day. An unusually large proportion of the milk business of the city is done by small dealers, and there is less of a tendency to concentration than is noticeable in some other places. Although 4 per cent of the dealers do one fourth of the entire business, the smallest dealers (69 per cent of the whole) do another fourth. Between these there are 9 per cent that do a fourth, and the remaining 18 per cent another fourth of the business. In other words, 13 per cent (the larger dealers) do one half the business, and the smaller dealers (87 per cent of all) do the other half.

Some of the small plants are open to criticism from the sanitary standpoint, a prominent fault being the intermingling of domestic and business life on account of the small business done. Yet all are constantly improving, and the good work of the health department is noticeable on every hand. All milk rooms are required to have clean cement floors, tidy, well-whitewashed walls, and good drainage, being connected with city sewers. Four per cent of the dealers score 90 or above; 16 per cent score 80 or above; 49 per cent score 70 or above; 25 per cent score 60 or above; 6 per cent score 50 or above.

Milk on reaching the dealers' plants has in the great majority of cases been from four to seven hours in transit, frequently without any refrigeration. It is usually cooled to some extent on the farm. It reaches the city plants between 10 and 12 o'clock a. m., and is cooled at once. In most cases this is done by placing the cans in tanks of ice water. Some of these tanks are primitive wooden boxes

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[1] The total number of separate premises in the District upon which cows are kept is 561.

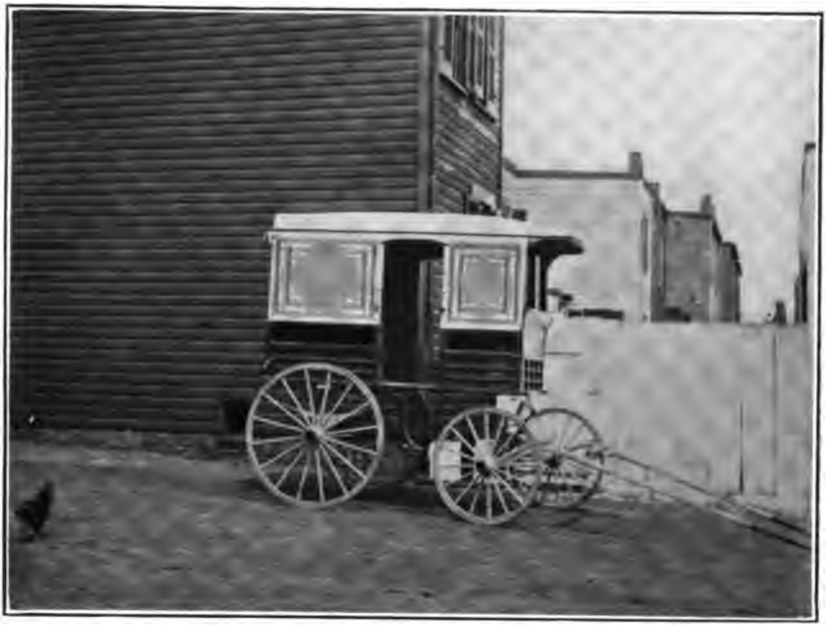

FIG. 1.—PLACE IN REAR OF BASEMENT, WITH ONLY ONE SMALL WINDOW, SHOWN AT REAR WHEEL OF WAGON.

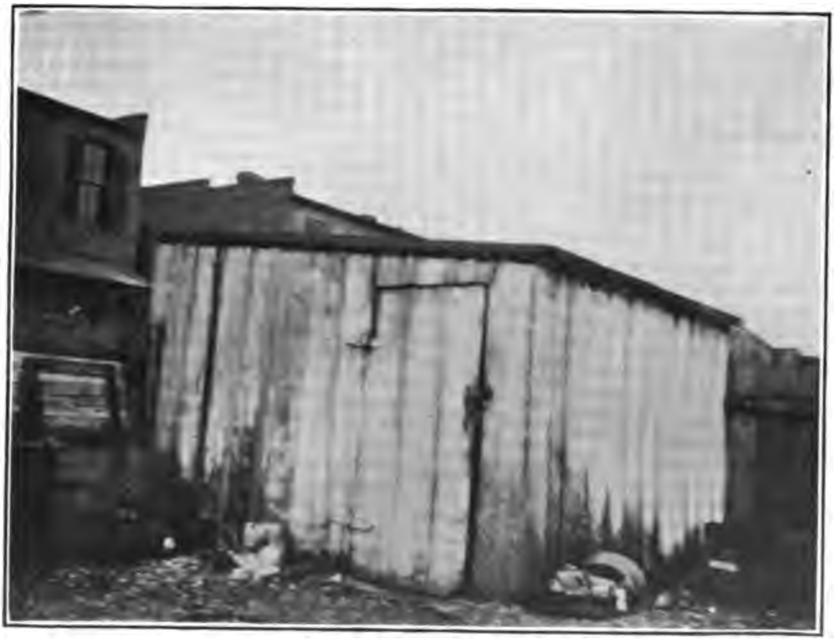

FIG. 2.—SMALL REAR SHED.

UNDESIRABLE WASHINGTON MILK PLANTS.

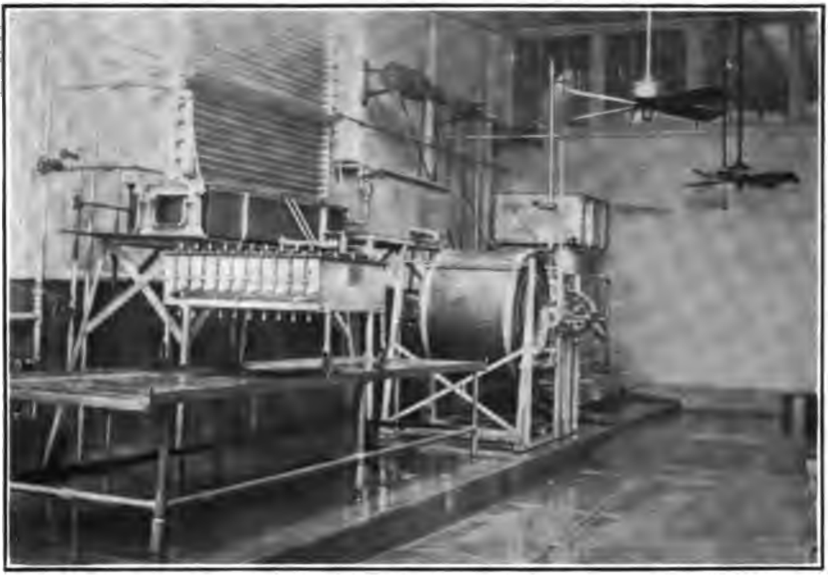

FIG. 1.—INTERIOR OF A LARGE, CLEAN, WELL-EQUIPPED PLANT.

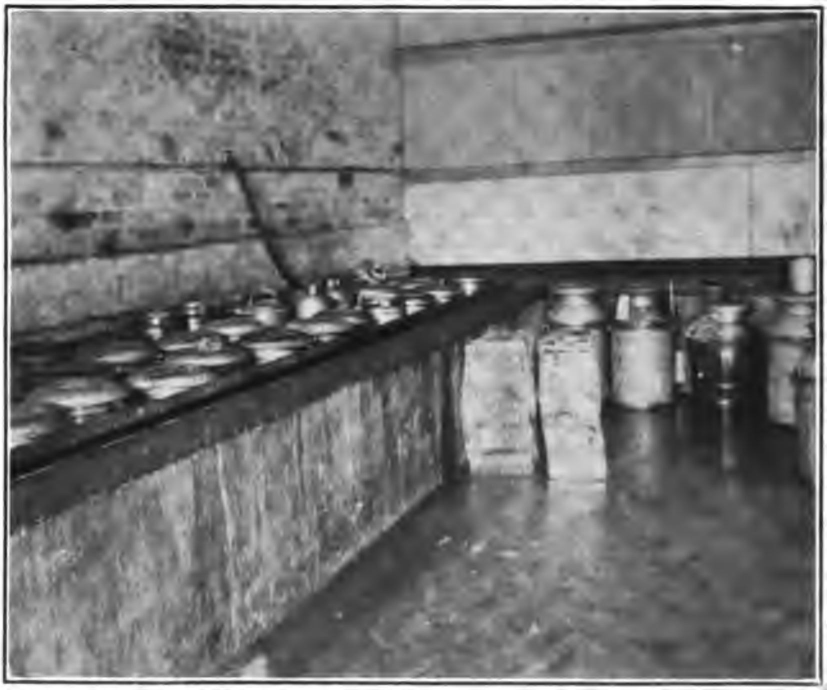

FIG. 2.—CEMENT COOLING TANK IN COLD-STORAGE ROOM OF LARGE PLANT.

VIEWS IN HIGH-CLASS WASHINGTON MILK PLANTS.

more or less worn and decayed, musty, and moldy. Others are clean and thoroughly constructed, made of cement with metal edges (Pl. VIII, fig. 2). After the milk is cooled it is bottled and then put in cold storage until time for the morning delivery. Only a few years ago most of the milk was bottled by hand, even in some of the largest establishments, but more bottling machines are being introduced. During the summer some dealers prefer to hold the milk on ice in the receiving cans and bottle early in the morning just before delivery. Icing on delivery wagons is not common. Some of the milkmen have machinery for filtering and pasteurizing, and some have refrigerating outfits; many have boilers furnishing an abundance of hot water and steam for cleansing purposes. The tendency to pasteurization is on the increase, and six of the largest dealers now pasteurize most of their product.

DEALERS' AND PRODUCERS' ORGANIZATIONS.

The milk dealers of the District have an organization known as the "Dairymen's Association of the District of Columbia, Maryland, and Virginia." The producers, of whom there are about 1,000, also have an organization called "Milk Producers' Association of Maryland, Virginia, and the District of Columbia."

WHOLESALE AND RETAIL PRICES.

The price to the farmers is made for six-months periods, November 1 and May 1. The ordinary summer price some time ago was 12½ cents and 13 cents a gallon, and the winter price 17 to 18 cents. During the winter of 1906–7 as high as 20 cents was paid in some instances. The wholesale price for the summer of 1909 was 14 to 16 cents a gallon, and the producers' association fixed a price of 22 cents for the winter of 1909–10. More is paid for milk produced and handled under special sanitary conditions. The farmers pay the freight, furnish the cans, and sell the milk "delivered in Washington in good condition." In dealings between the producer and the dealer there are as a rule no written contracts, only oral agreements. In most cases the trade is for a stated amount daily.

The retail price ranges from 8 to 10 cents a quart, according to quality. Ordinary milk sells at 8 cents in summer and 9 cents in winter.

LAWS AND INSPECTION.

A milk law enacted by Congress for the District of Columbia became effective in March, 1895, and milk inspection in the District dates from that year.

Concerning this law Dr. William C. Woodward, the health officer, says:[1]

> Experience soon revealed defects, and efforts were promptly made to correct them. * * * The result has, however, not been encouraging. Bills introduced on recommendation of the health officer and receiving the indorsement of the Commissioners and of the Medical Society of the District of Columbia have been uniformly opposed by milk interests. The fight to obtain better legislation to regulate the sale of milk has always been carried to the committee room at the Capitol, and the fight has always been lost.

In spite of its defects, the law has proved very helpful in raising the quality of the District milk supply and reducing infant mortality.

The health department had no milk bacteriologist until 1910 and up to that time had made no bacteriological inspections of milk, while many cities had had a bacteriological laboratory for several years and considered a bacteriologist fully as important as a chemist in the inspection of milk. The appropriation act approved May, 1910, provided $500 for the equipment and maintenance of such a laboratory and authorized the bacteriologist who had been employed for contagious diseases service to undertake the bacteriological examination of milk and the water supply of dairy farms.

The law of 1895 contains many provisions ordinarily found in such laws. A system of licenses or permits is provided for. A producer who retails his own product is required to have two permits, one as a dealer and one as a producer. If he lives outside of the District his producer's permit is known as an "importer's" permit. The dealer's permit is known as a "dairy" permit. The law contains the unusual provision that grocers, bakers, and others selling milk shall post the names of dairymen from whom the milk is obtained, but does not require them to have a license. A person applying for a license can commence business at once pending the issuing of the license.

A general food law for the District of Columbia, passed a few years after the milk-inspection law, establishes a milk standard of 3½ per cent of fat, 9 per cent of solids not fat, and 12½ per cent of total solids.

The standard for cream is 20 per cent fat, 18 per cent being the enforceable figure. This quality is usually called table cream. Double cream has 40 per cent fat. Not much of the latter is sold, although considerable of the shipments from a distance have 40 per cent fat to save freight and on arrival it is reduced with milk to standard or 20 per cent cream.

By an order issued November 5, 1910, relative to applications for new permits if all cattle on the dairy farm to which any such application relates are not free from tuberculosis as shown by the tuberculin test the application will be rejected.

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[1] "The municipal regulation of the milk supply of the District of Columbia," in Bulletin 56 of the Hygienic Laboratory, Public Health and Marine-Hospital Service, p. 747, 1909.

The enforcement of the dairy laws is placed in the control of the District health department, but no provision is made for a distinctive milk division with a chief responsible for the milk and dairy inspection. In the roll of officers and employees of the health department there is no indication of anyone giving his exclusive attention to milk work. The department has 17 "sanitary and food inspectors," and one of these gives most of his time to inspecting city milk plants, while another is assigned to collecting samples. There are five veterinarians acting as inspectors who give most of their time to farm inspection, although they have to do also with rabies, glanders, and abattoir inspection. They are also permitted to engage in private practice on account of their small salaries, hence their undivided energies can not be given to dairy work. The producing territory is divided into districts having from 123 to 205 milk-producing farms in each, according to their location, and a veterinary inspector has charge of each district. The general supervision of the country work is intrusted to the chief clerk in the health office, and that of the city work to the chief inspector. The general chemist of the health office makes all of the milk analyses.

The national pure-food law gives the United States Department of Agriculture authority to enforce it in the District of Columbia, in the Territories, and in interstate and foreign commerce. The District health officer and his assistants have therefore been appointed collaborating officials of the Department of Agriculture, to collect samples, conduct hearings, and bring cases to the attention of the district attorney. The pure-food law also requires that analyses shall be made under the general direction of the Bureau of Chemistry. Consequently one of the chemists in the office of the District health officer has been appointed a collaborating chemist of the Bureau of Chemistry.

The District health department has done commendable work in milk inspection with inadequate powers and means and under adverse conditions. The health officer himself has frequently called attention to the weaknesses of the situation. In his report for 1907 he said:

> The work that the service has accomplished has been well done, but it could have been more intelligently and better done if provision had been made whereby some one person in the department could be, by the health officer, held responsible for the proper conduct of the service.

The expense of milk inspection in the District can only be approximated. Salaries aggregating $10,900 are paid to inspectors who give most of their time to milk work, and it is estimated that a proper pro rata of other salaries and expenses chargeable to the milk service is $4,900, making the total expense of milk inspection $15,800.

Congress has given the health department power to make regulations relative to water supply, drainage, ventilation, air space, floor space, and cleaning of milk plants and dairies within the District. Besides this, Congress requires that persons who bring or send milk into the District shall have a permit, which the health officer is given power to withhold or revoke for cause. He has therefore a lever with which he can do much in improving the methods of milk production.

A most remarkable fall in the death rate among infants from diarrheal diseases began with the establishment of milk inspection in 1895 and has progressed as the efficiency of that service has been increased.

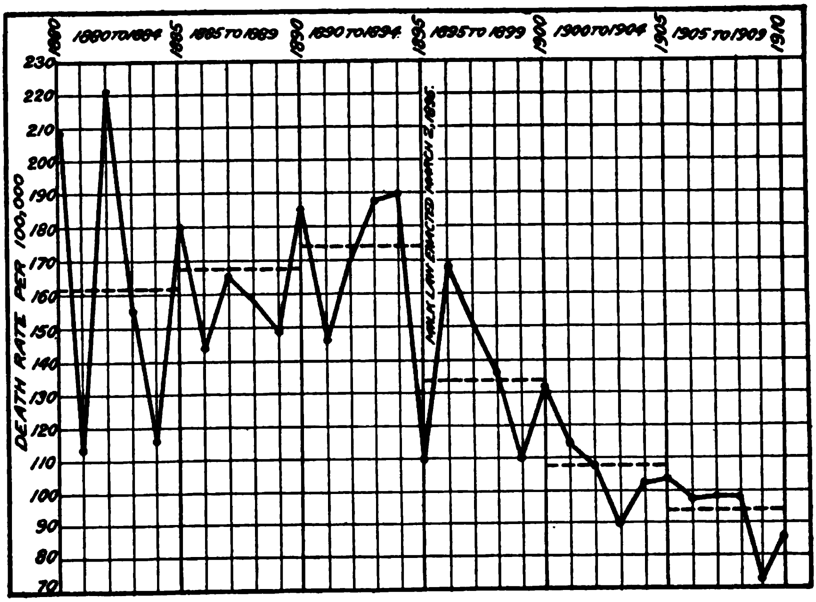

FIG. 6.—Chart showing decrease in death rate from diarrhea and enteritis in children under 2 years of age in the District of Columbia, following enactment of milk law of 1895. Dotted lines show averages for five-year periods.

This decrease in mortality is graphically shown in figure 6, which is reproduced from Dr. Woodward's paper in Hygienic Laboratory Bulletin 56 and is brought down to 1910 with information furnished by the District health department.

The improvement in the chemical quality of milk samples collected about the city is shown by the following table, which gives the percentage of samples below the standard during a period of four years:

	Per cent.
1903–4	27.50
1904–5	20.32
1905–6	12.37
1906–7	10.95
1907–8	16.41

During the fiscal year 1906–7, 883 farms were inspected, on an average, 4.4 times each; 4,960 samples of milk and cream were taken and analyzed; 77 milk plants were inspected an average of 22.8 times; 289 cases were prosecuted; and 139 requests for producers' permits were refused. During the year 1908–9 there were 4,294 inspections of farms upon which were 16,116 cows; 6,659 samples of milk and 101 samples of cream were collected and 135 cases were prosecuted, of which 130 resulted in convictions. Of the samples analyzed, 412 of milk and 45 of cream were found to be adulterated, and 243 of milk were below the standard (3.5 per cent) in fat.

A MILK-STANDARD DECISION.

As there is considerable agitation over the milk standard in various places, a decision of the Court of Appeals of the District of Columbia, rendered November 5, 1903, is of interest. The defense offered to show that 3.5 per cent of fat, "the standard prescribed, was unreasonably high and could not by ordinary care be maintained through all seasons of the year." The trial court refused to admit this evidence. The court of appeals sustained this ruling, saying:

> If the proposition of the defendant were sustained, the question of the reasonableness of the statute would be one of fact for the jury, and we should likely have different juries determining the question in different ways.

The court also said:

> To declare an act of Congress unreasonable and oppressive, and therefore void, is a power that the courts can not exercise except when the provision of the statute is shown to be plainly violative of some provision of the Constitution. The subject matter of the act of 1898 [a statutory milk standard] is plainly within the power of Congress, and the courts can not amend or modify any of the provisions of that act so as to bring them within what may seem to be reasonable bounds. They can not examine a question as expedient or inexpedient, as politic or impolitic.

The court also held that in case of food laws the question of intent is not involved; the party making a sale is bound at his peril to know what he is selling.

FEDERAL COOPERATION.

The milk supply of the District receives considerable attention from the Federal Government, and Federal officers are of material assistance to the District authorities. The Public Health and Marine-Hospital Service made an exhaustive study during the summer of 1906 of the prevalence of typhoid fever. (See Hygienic Laboratory Bulletin 34.) Bulletin 56, "Milk and its Relation to Public Health," from the same laboratory also contains the result of much investigation into local conditions.

The Bureau of Animal Industry of the Department of Agriculture has also been greatly interested in the District milk supply and has issued Circulars 111, 114, and 153 relating to the local conditions.

Since 1907 the bureau has gratuitously tested with tuberculin cows supplying the District with milk, the tests being made upon request of the owners of the herds. Out of 4,200 tests (including 1,729 retests) 12.93 per cent of the cows proved tuberculous. Of the animals tested for the first time 15.25 per cent reacted, and 9.16 per cent of those retested reacted. These tests include many herds in Maryland and Virginia.

In the latter part of November, 1909, the bureau began the systematic testing of all cattle in the District of Columbia under a cooperative arrangement with the District Commissioners and in accordance with an order of the commissioners, approved by the Secretary of Agriculture, issued November 26, 1909, for the suppression and prevention of tuberculosis in cattle within the District of Columbia. Under this order the tuberculin testing of all cows within the District has now been accomplished, the reactors have been slaughtered, the owners indemnified for the loss of tuberculous animals, and the premises disinfected. The number of cattle tested was 1,701, of which 321, or 18.87 per cent, reacted and were slaughtered, lesions of tuberculosis being found on post-mortem examination in all but 5 of the carcasses.[1] Cattle are being retested after the lapse of six months in order to detect any cases of tuberculosis that may have developed since previous tests. All dairy cattle brought into the District must be shown by the tuberculin test to be free from tuberculosis.

Material assistance has also been rendered by the bureau in investigating a recent outbreak of typhoid fever. The Dairy Division of the bureau has examined and scored every dairy farm contributing to the District supply and every dealer's establishment in the District and has cooperated with the District inspectors in the use of the score card.

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[1] A report of this work is made by Dr. R. W. Hickman, chief of the Quarantine Division of the Bureau of Animal Industry, in a paper in the Yearbook of the United States Department of Agriculture for 1910.

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Issued August 14, 1911.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY.—BULLETIN 139.
A. D. MELVIN, CHIEF OF BUREAU.

THE NUTRITIVE VALUE OF THE NONPROTEIN OF FEEDING STUFFS.

BY

HENRY PRENTISS ARMSBY, PH. D., LL. D.,
Director of the Institute of Animal Nutrition of The Pennsylvania State College; Expert in Animal Nutrition, Bureau of Animal Industry.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1911.

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LETTER OF TRANSMITTAL.

UNITED STATES DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., May 8, 1911.

SIR: I have the honor to transmit herewith a manuscript on "The Nutritive Value of the Nonprotein of Feeding Stuffs," by Dr. Henry Prentiss Armsby, who is in charge of the cooperative investigations in animal nutrition by this bureau and the Institute of Animal Nutrition of the Pennsylvania State College. Preliminary to writing a paper dealing with maintenance requirements in feeding animals he has found it necessary to consider the value of the nonprotein nitrogenous substances in the ration. He has therefore in the accompanying manuscript reviewed the literature of investigations on the latter subject and summarized the results, and in conclusion has discussed their bearing. I respectfully recommend the publication of this paper as a bulletin of this bureau.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

Page.
Introduction 5
Experiments on carnivora 6
Experiments on omnivora 12
Experiments on herbivora 12
  Behavior of nonproteins in digestive tract of herbivora 14
  Nutritive value of nonprotein for herbivora 28
    Nonprotein a source of protein 28
    Effect of nonprotein on total production 38
  Direct utilization of ammonium salts 44
Résumé 45
Conclusions 46
  Value for maintenance of protein tissues 46
  Value for production 47
  The computation of rations 47

THE NUTRITIVE VALUE OF THE NONPROTEIN OF FEEDING STUFFS.

INTRODUCTION.

It is well known that the nitrogenous constituents of feeding stuffs comprise, besides the true proteins, numerous other compounds of the most varied nature, including alkaloids, nitrogenous glucosids, amino acids and amids, phosphatids, nitrates, ammonium salts, etc., so that in the aggregate a not inconsiderable proportion of the nitrogen supply of herbivorous animals is derived from these substances. All these diverse nitrogenous compounds have been for convenience grouped under the name "nonprotein." The name, of course, is an abbreviation for nonprotein nitrogenous matters, and, as the foregoing partial enumeration shows, the group is very heterogeneous in its nature.

Alkaloids and nitrogenous glucosids do not appear to be especially abundant in the ordinary feeding stuffs of domestic animals, and where they do occur are distinguished rather by specific physiological effects than by their nutritive value in the ordinary sense. As regards the nutritive value of the phosphatids, comparatively little is known, although it is claimed that the lecithins have a stimulating effect upon growth. Of all the groups of nonnitrogenous substances above enumerated, the amino acids and amids appear to be most abundant. Moreover, they are of special interest because they are products of the protein metabolism of the plant and are to a considerable extent identical with the protein cleavage products which appear to play such a large rôle in animal nutrition. From a practical standpoint, then, the question of the nutritive value of nonproteins is largely identical with that of the nutritive value of the so-called "amids." This question is considered in the present publication solely from the standpoint of their value for the maintenance or production of body protein, without reference to their value as sources of energy.

It has been shown by physiologists that the animal undoubtedly has the power to build up body proteins out of the comparatively simple cleavage products resulting from the digestion of food proteins. It is natural to assume, therefore, that when similar cleavage products are found in feeding stuffs they are capable of undergoing the same chemical reactions in the body as if they arose from the digestive cleavage of protein. Looking at the question of the nutritive value of the nonprotein from this point of view, it is apparent that the question is to a considerable extent similar to that of the relative values of different proteins. Just as the proportions of the different amino acids, etc., yielded by different proteins vary, so do the proportions of the similar substances found in the nonprotein of different feeding stuffs, while neither the proportions nor the specific compounds are identical in proteins and nonproteins. Evidently, then, it is futile to seek to establish any definite ratio between protein and nonprotein as to their value to the organism, because both of them, but especially the latter, are in this respect more or less variable and indefinite conceptions. The failure to recognize this fact is responsible for not a little of the existing confusion of thought on this question. Thus, many of the earlier investigations of the nutritive value of nonproteins[1] were made upon single amids or amino acids, notably on asparagin, largely because the latter occurs rather abundantly in plants and is readily obtained reasonably pure, although it is not itself a constituent of the protein molecule.[2] In these earlier experiments numerous investigators showed that various single amino acids and amids are katabolized in the animal body, their nitrogen reappearing as urea, although Völtz[3] has lately claimed, contrary to earlier results by Andrlik, Velich, and Stanek,[4] that betain is an exception. As regards their ability to replace the protein of the food, however, or to maintain protein tissue, these experiments indicated a marked apparent difference between carnivorous and omnivorous animals on the one hand and the herbivora, particularly ruminants, on the other.

EXPERIMENTS ON CARNIVORA.

The earlier experiments made upon carnivora as a rule failed to show that amids could to any degree serve to protect the protein tissue of the body from katabolism. More recent experiments have, upon the whole, confirmed these results. While numerous investigations have shown beyond a doubt that the body has the power to build up protein from the mixture of at least very simple cleavage products obtained by prolonged enzym hydrolysis, or even by acid hydrolysis,[5] experiments in which single amino acids or even three or four, or the mixture contained in vegetable extracts, have been fed have failed to establish satisfactorily the ability of the organism to form protein from them. The principal investigations on this question have been by Völtz, Lehmann, Rosenfeld, and Müller.

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[1] Compare Armsby, Henry P. The Principles of Animal Nutrition. Third edition, revised, New York, 1908, pp. 52–58.

[2] Asparagin is the amid of aspartic acid, which is one of the cleavage products of all proteins thus far investigated.

[3] Völtz, W. Untersuchungen über die Verwertung des Betains durch den Wiederkäuer (Schaf). Archiv für die gesammte Physiologie, Band 116, Heft, 5–6, pp. 307–333. Bonn, 1907.

[4] Zeitschrift Zuckerindustrie in Böhmen, Band 27, p. 14.

[5] Abderhalden, Emil, and Frank, Oskar. Weiterer Beitrag zur Frage nach der Verwertung von tief abgebautem Eiweiss im tierischen Organismus. XII Mitteilung. Zeitschrift für physiologische Chemie. Band 64, Heft 2–3, pp. 158–163. Strassburg, 1910.

Völtz[1] has reported three series of experiments upon dogs. In the first he compared the effect of adding to the basal ration consumed by a mature dog, on the one hand, 1 gram of nitrogen in the form of protein of various kinds and on the other hand one-half gram in the form of protein and the remainder as asparagin. As regards the relative values of the proteins, the experiments are open to the criticism that the protein content of the basal ration was too high. The latter contained 0.37 to 0.75 gram total nitrogen per kilogram live weight, as compared with the 0.2 to 0.3 gram per kilogram which apparently suffices for maintenance.[2] In other words, surplus protein was being katabolized in the body in these experiments. It is not surprising to find, therefore, that the effects upon the nitrogen balance of adding more protein to the ration were irregular and difficult to interpret. The asparagin, however, in every case was found to be inferior to protein in its power of maintaining or increasing the body protein. It may be noted also in passing that the asparagin increased the nitrogen content of the feces. If we may judge from the results upon herbivora, to be considered subsequently, this may be ascribed to an increase in the so-called metabolic products, especially mucus and epithelial detritus.

In the second series of experiments, also on a mature dog, a basal ration containing about 0.75 gram nitrogen per kilogram body weight was fed in the first and last periods. In the remaining periods 1 gram of nitrogen in various forms (asparagin, ammonium acetate, acetamid, and glycocol) was added to the basal ration. Völtz bases his conclusions upon the average of the last 7 days of the 10-day periods. His results show during the periods of nonprotein feeding an excess of nitrogen in the urine over the average of the basal period greater than the amount of nonprotein nitrogen added to the ration. That is, the nonproteins diminished the gain of nitrogen by the body. In his final table Völtz assumes the fecal nitrogen for each period as equal to that of the first, disregarding an observed steady increase in the subsequent periods, including the last basal ration. In this manner he computes that in period 6, in which a mixture of the nonproteins used in the preceding periods was fed, the nitrogen katabolism remained practically the same as in the basal period, and hence concludes that the mixture was more effective in this respect than its ingredients separately, and argues that experiments upon a single amid or amino acid are inconclusive as regards the value of the mixed nonproteins of natural products. The general correctness of this point of view was pointed out in the introduction, but Kellner[1] has called attention to the rather remarkable nature of Völtz's calculations, and has shown that when the actual analytical results are made the basis of the calculation it appears that the animal, which in the first period was gaining nitrogen, was steadily approaching a condition of nitrogen equilibrium in the last period, and that the addition of the nonproteins to the ration produced no distinct effect.

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[1] Völtz, W. Über den Einfluss verschiedener Eiweisskörper und einiger Derivate derselben auf den Stickstoffumsatz, mit besonderer Berücksichtigung des Asparagins. Archiv für die gesammte Physiologie, Band 107, Heft 7–9, pp. 360–414. Bonn, 1905.

Über den Einfluss des Lezithins auf den Eiweissumsatz ohne gleichzeitige Asparaginzufuhr und bei Gegenwart dieses Amids. Zeitschrift für die gesammte Physiologie, Band 107, Heft 7–9, pp. 415–425. Bonn, 1905.

Über das Verhalten einiger Amidsubstanzen allein und im Gemisch im Stoffwechsel der Karnivoren, Zeitschrift für die gesammte Physiologie, Band 112, Heft 7–8, pp. 413–438. Bonn, 1906.

Völtz, W., and Yakuwa, G. Über die Verwertung verschiedener Amidsubstanzen durch Carnivoren. Zeitchrift für die gesammte Physiologie, Band 121, Heft 3–4, pp. 117–149. Bonn, 1908.

[2] Compare Armsby, Principles of Animal Nutrition, p. 143, 1908; and Chittenden, The Nutrition of Man, Chapter VII, 1907.

In his third series of experiments, upon one growing and two mature dogs, Völtz followed the same general plan as in the previous series, but used five-day periods, alternating with similar periods on the basal ration. As before, 1 gram of nitrogen in the same four forms was added to a basal ration containing, in the case of the mature animals, 0.58 to 0.73 gram, and in that of the growing animals from 0.43 to 0.46 gram nitrogen per kilogram body weight; that is, materially more than the minimum protein requirement. In these experiments, contrary to the earlier ones, the amount of nitrogen contained in the feces was slightly less instead of greater in the periods in which the nonproteins were fed. The results are corrected for the effect of the nonproteins upon the nitrogen excretion at the beginning of the following basal period from the data for the daily nitrogen excretion. Thus corrected, the periods in which acetamid, ammonium acetate, and a mixture of nonproteins were consumed showed a considerable retention of nonprotein nitrogen, while the periods with asparagin and with glycocol failed to do so. It may be noted that dog No. 3 (mature) showed a distinct tendency toward a gain of nitrogen even on the basal ration, while dog No. 2 (immature) did not.

Rosenfeld,[2] at the suggestion of C. Lehmann, investigated the influence of the bulk of food upon the utilization of asparagin by the dog by cooking very finely ground hay with the remaining feed. He found in the hay periods a considerable retention of the nitrogen of the asparagin added to the ration, while when albumin was substituted for asparagin the retention was less. He concludes that under these conditions there is either an action of the ferment organisms similar to that occurring in herbivora[1] or that some substances resorbed from the hay facilitate the utilization of the asparagin.

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[1] Kellner, O. Zur Kenntniss der Wirkung nicht eiweissartiger Stickstoffverbindungen auf den Stickstoffumsatz im Tierkörper. Archiv für die gesammte Physiologie, Band 113, Heft 7-8, pp. 480-486. Bonn, 1906. See p. 484.

[2] Cited by Völtz, Archiv für die gesammte Physiologie, Band 107, Heft 7-9, p. 365.

Lehmann[2] reports experiments performed by Rosenfeld on another phase of the same general idea, viz, that the rate of resorption may materially affect the nutritive value of nonprotein. He points out that in experiments upon carnivorous animals soluble nonproteins (usually asparagin) have commonly been given in a single dose so that they were rapidly resorbed, while with herbivorous animals, on the contrary, the resorption from ordinary feeding stuffs would be relatively slower. In the first case, therefore, the system would be more or less flooded temporarily with the nonprotein, which would presumably be subject to rapid nitrogen cleavage; and Lehmann cites Graffenberger's experiments[3] in illustration of this effect. To test the truth of this view, Lehmann prepared a mixture of asparagin and a solution of celluloid and allowed it to dry out in small grains. This coated asparagin was compared with untreated material and with blood albumin, it being shown by preliminary trials that the solution of the asparagin was rendered less rapid by the treatment, but that it was completely digested by the animal. The subject, a dog, received a basal ration, consisting of meat, rice, lard, and ash ingredients, which contained 0.55 gram nitrogen per kilogram of live weight. No statement of the energy content of the ration is made, but the fact that the nitrogen katabolism seems to have steadily increased in the basal periods suggests an insufficient supply. Increasing amounts of nitrogen in the three forms mentioned were added in successive three-day periods in amounts ranging from 1 to 2 grams, a period upon a basal ration preceding each of the three series of trials.

In summarizing his results Lehmann compares for each three-day period the excess of nitrogen digested over that digested in the period immediately preceding with the excess of nitrogen found in the urine of the same three days. Compared in this way he finds that the free asparagin increased slightly the loss of nitrogen from the body, while the coated asparagin maintained nitrogen equilibrium and the blood albumin caused a slight gain. Neglecting the minute amounts of nitrogen given off in the hair, the results may also be summarized as in the following table:

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[1] Compare p. 13.

[2] Lehmann, C. Beiträge zur Kenntniss der Wirkung des Asparagins auf den Stickstoffumsatz im Thierkörper. Archiv für die gesammte Physiologie, Band 112, Heft 7-8, pp. 339-351. Bonn, 1906.

[3] Graffenberger, L. Versuche zur Feststellung des zeitlichen Ablaufes der Zersetzung von Fibrin, Leim, Pepton und Asparagin im menschlichen Organismus. Zeitschrift für Biologie, Band 28, pp. 318-344. München and Leipzig, 1891.

Average nitrogen per day—Lehmann's experiments.

Items.	Digested.	In urine.	Gain or loss.
	Grams.	*Grams.*	*Grams.*
Basal period	5.13	4.99	+0.14
Asparagin, coated	6.55	6.53	+0.02
Basal period	5.11	5.10	+0.01
Asparagin, free	6.66	6.85	−0.19
Basal period	5.11	5.19	−0.08
Blood albumin	6.43	6.40	+0.03
Basal period	5.14	5.28	−0.14

It appears from these results that the asparagin had a tendency to increase the breaking down of nitrogenous body material, but the differences hardly seem very significant. Kellner[1] has criticized Lehmann's conclusions because he failed to take due account of the lag in the excretion of urinary nitrogen and of the gradual increase in the nitrogen katabolism upon the basal ration. He shows from Lehmann's data that some of the nitrogen added to the basal ration appeared in the urine on the first day or two of the following basal period (these days are not included in the data of the table). Recalculating the results from this point of view, Kellner computes that both forms of asparagin had an equal effect in materially increasing the nitrogen katabolism, while the albumin, on the other hand, diminished it somewhat. In reply Lehmann[2] denies that Kellner is justified in assuming an increasing nitrogen katabolism for the basal periods and thus estimating what the nitrogen balance would have been in each period without the added nitrogen. By comparing the actual figures Lehmann shows a distinct negative balance upon the free asparagin as compared with a slight positive balance on the coated asparagin and the albumin.

Müller[3] has repeated Lehmann's experiments with coated and uncoated asparagin, with the difference that he added more nitrogen to the basal ration in proportion to the live weight than did Lehmann and also added sufficient nonnitrogenous material to compensate for the difference in energy between asparagin and an amount of albumin containing the same quantity of nitrogen. On the basal periods the ration contained 0.56 to 0.64 gram nitrogen per kilogram live weight, and the animal was quite exactly in nitrogen equilibrium. After correcting the observed results in each period for the influence of the lag of nitrogen excretion as shown by the succeeding basal period he finds some retention of the added nitrogen in all cases, but notably less in the case of the free asparagin. Including a correction for the small gain or loss in the basal ration, he computes a retention of the following amounts of nitrogen per day:

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[1] Kellner, O. Zur Kenntnis der Wirkung nicht eiweissartiger Stickstoffverbindungen auf den Stickstoffumsatz im Tierkörper. Archiv für die gesammte Physiologie, Band 113, Heft 7-8, pp. 480–486. See p. 484. Bonn, 1906.

[2] Lehmann, C. Nochmals zur Wirkung des Asparagins auf den Stickstoffumsatz im Tierkörper. Archiv für die gesammte Physiologie, Band 115, Heft, 115, pp. 448–451. Bonn, 1906.

[3] Müller, Max. Weitere Untersuchungen über die Wirkung des Asparagins auf den Stickstoffumsatz und Ansatz des Tierkörpers. Archiv für die gesammte Physiologie, Band 117, Heft 10-12, pp. 497–537. Bonn, 1907.

	Gram.
Asparagin, coated	0.35
Asparagin, uncoated	.18
Blood albumin	.35
Dextrin	.06

In a subsequent investigation [1] Müller has compared the effect of blood albumin with that of the mixed nitrogenous material contained in an aqueous extract of hay. A mature dog received a basal ration containing 0.55 gram nitrogen per kilogram live weight. To this were added in subsequent periods equal amounts of nitrogen in the forms of blood albumin and of hay extract, no addition of nonnitrogenous material being made and no correction being made for the lag in the nitrogen excretion. A small plus balance of nitrogen was observed in both cases, but it was much smaller with the hay extract than with the blood albumin, being, indeed, almost or quite negligible.

Müller's experiments have also been criticized by Kellner [2] on the ground that in the second series the nitrogen lag was not taken account of, while he suspects analytical errors in the nitrogen determinations of both series (although this is denied by Müller [3]), while Friedländer [4] regards the differences observed by Müller as within the limits of analytical error and also criticizes his short periods.

Quite aside, however, from the points raised by Kellner and others, there is one feature of all these experiments which renders their results inconclusive, viz, the fact that, as already pointed out, the nitrogenous substances to be tested were *added* to a basal ration which already contained a surplus of protein over the maintenance requirement. When the animal was in nitrogen equilibrium with the basal ration, therefore, the nitrogenous cleavage products arising from the digestion of the protein were doubtless being deamidized to a considerable extent and their nitrogen excreted as urea. Such being the case, while additional protein might cause more or less gain of nitrogen for a time, additional nonprotein might easily produce indirectly a similar effect, without implying any formation of protein from it, simply by taking the place of some of the cleavage products whose nitrogen was previously split off. In other words, it is possible that the nitrogen stored up in the body had its origin in the surplus protein of the food and not in the nonprotein added. The fact that the retention of nitrogen when nonprotein was fed was frequently less than that when a corresponding amount of protein was given may possibly be explained by the fact that one or two single amino acids could not fully replace in this respect the mixture arising from the digestion of the protein of the food. Taking all these points into consideration, we seem warranted in concluding that it has not yet been satisfactorily shown that carnivorous animals can produce body protein either from one or a few of the amino acids or from the mixture of nonproteins contained in the vegetable products thus far investigated.

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[1] Müller, Max. Untersuchungen über die Nährwirkung im Heu enthaltener Nichteiweisse. Journal für Landwirthschaft, Band 55, pp. 123–141. Berlin, 1907.

[2] Kellner, O. Notiz betreffend die Nährwirkung des Asparagins. Archiv für die gesammte Physiologie, Band 118, Heft 11–12, pp. 641–642. Bonn, 1907.

Kellner, O. Untersuchungen über die Nährwirkung der im Heu enhaltenen nichteiweissartigen Stickstoffverbindungen. Journal für Landwirtschaft, Band 56, pp. 49–52. Berlin, 1908.

[3] Müller, Max. Richtigstellung der von O. Kellner gemachten kritischen Bemerkungen. Journal für Landwirtschaft, Jahrgang 56, pp. 193–194. Berlin, 1908.

[4] Friedländer, Konrad. Zur Frage des Eiweissersatzes durch Amide. Die landwirtschaftlichen Versuchs-Stationen, Band 67, pp. 283–312. Berlin, 1907. See pp. 289–292.

EXPERIMENTS ON OMNIVORA.

To a number of earlier investigations on omnivorous animals, mostly rats and mice, may be added more recent experiments by Henriques and Hansen [1] upon rats, directed, like the earlier ones, to a somewhat different aspect of the problem than that studied in the foregoing experiments on carnivora. They investigated the question whether asparagin or the mixture of nonprotein nitrogenous materials found in various vegetable substances (potatoes, roots, and seedlings of beans and barley), when constituting the sole source of protein, were capable of maintaining the protein tissue of the body. Their results fully confirmed the earlier ones of Politis and of Gabriel and showed that under these circumstances the nonprotein can not perform the functions of protein. A continuous loss of nitrogen from the body was observed, which was substantially at the same rate as when only nonnitrogenous nutrients were consumed.

EXPERIMENTS ON HERBIVORA.

But while there is no satisfactory evidence that either single nonproteins or the mixtures of them found in vegetable products are available to either carnivora or omnivora as a source of protein, with herbivora, as previously indicated, the case is different.[2] With the latter class of animals a considerable number of experiments are on record, of which Weiske's are the earliest, which have shown that asparagin added to a ration poor in protein is able partially to replace the latter. Zuntz appears to have been the first to advance the idea that this marked difference between the two classes of animals might be due to the difference in their digestive processes and particularly to the great development of organized ferments in the digestive tract of herbivora. He suggests that soluble nonprotein introduced into the digestive canal of herbivora may be used as nitrogenous food by the organisms in preference to the less soluble proteins, particularly in the first stomach of ruminants, before the digestion of the proteins begins, so that the latter are to a greater or less extent protected from destruction, while it is possible that the nonproteins are thus synthesized to protein by the organisms and later digested by the animal.

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[1] Henriques, V., and Hansen, C. Über die Bedeutung der sogenannten Pflanzenamide für den Stickstoffumsatz im tierischen Organismus. Zeitschrift für physiologische Chemie, Band 54, pp. 169–187. Strassburg, 1907–8.

[2] Compare Armsby, Principles of Animal Nutrition, pp. 53–58. New York, 1908.

The validity of this suggestion was confirmed by Kellner[1] in experiments upon lambs, in which the gain of nitrogen by the animal upon a ration poor in protein was very notably increased by the addition to the ration not only of asparagin, but also of ammonium acetate, which it has not been supposed that the body tissues can synthesize to protein. He showed also that this effect was accompanied by an increased digestibility of the crude fiber and nitrogen-free extract of the ration, presumably due to the greater activity of the micro-organisms under the influence of the soluble nitrogenous food. Tryniszewsky also observed a similar effect upon the digestibility of the nonnitrogenous ingredients of the feed, although the effect upon the nitrogen balance was less decided.

This view, which has been generally accepted, regards the value of the nonprotein in the feed of herbivora as indirect, due to its protecting protein from destruction. The multiplication of organisms under the influence of an increased supply of nonprotein nitrogen has, however, another aspect, as appears when we inquire what becomes of the nitrogen which they assimilate. Presumably it becomes part of the protoplasm of the organisms and in this way may produce one of two effects. If any of the nitrogen of the feces has its origin in the nonprotein of the feed—that is, if the bacterial protein formed from the latter is indigestible—it is necessary to modify considerably the ordinary interpretation of digestion experiments upon rations containing nonproteins. Hitherto the latter, being soluble in water, have been regarded as totally digestible. If, however, part of the fecal nitrogen is derived from them, the digestion experiment as ordinarily computed gives too high a result for the resorbed nonprotein and correspondingly too low a result for the resorbed protein, and this leads to ascribing to the former nutritive effects really due to the latter.

On the other hand, if the bacterial protein formed from the nonproteins is digestible, we have in the activity of these organisms in the digestive tract a means of converting nonprotein into available protein, and so virtually adding to the protein supply in the food by a sort of symbiosis. It may be remarked that the presence of bacteria in the feces does not necessarily disprove this, since the latter may readily come from the lower part of the alimentary canal.

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[1] Kellner, O. Untersuchungen über den Einfluss des Asparagins und Ammoniaks auf den Eiweissumsatz der Wiederkäuer. Zeitschrift für Biologie, Band 39, pp. 313-376. München und Leipzig, 1900.

Since the interpretation of the results of feeding experiments upon herbivora must be materially affected by the question of the fate of the nonprotein in the digestive tract, it seems necessary to consider this aspect of the question first.

BEHAVIOR OF NONPROTEINS IN DIGESTIVE TRACT OF HERBIVORA.

There are a considerable number of experiments on record in which the protein of the feces has been distinctly increased by feeding materials containing much nonprotein nitrogen, and this has been interpreted as indicating the formation by bacteria in the digestive tract of indigestible nitrogenous compounds. In other cases, however, scarcely any such effect has been observed.

In the early experiments of Weiske, as well as in the later ones of Chomsky, more or less increase in the total nitrogen of the feces was observed to result when asparagin was fed. In Kellner's experiments upon lambs, just referred to, asparagin was substituted for an equal weight of starch in the first, third, and fourth series, while in the second and third periods of the second series ammonium acetate and asparagin, respectively, were added to the basal ration. The total excretion of fecal nitrogen was as follows:

Excretion of fecal nitrogen—Kellner's experiments.

Items.	Lamb I.	Lamb II.
Series I:	*Grams.*	*Grams.*
Period 1, basal ration	6.51	6.32
Period 2, asparagin substituted	5.54	5.77
Series II:		
Period 1, basal ration	6.09	6.17
Period 2, ammonium acetate added	6.63	6.16
Period 3, asparagin added	6.25	5.63
Series III:		
Period 1, basal ration	7.17	6.95
Period 2, asparagin substituted	7.08	6.85
Series IV:		
Period 1, basal ration	7.66	6.09
Period 2, asparagin substituted	7.70	6.28
Period 3, basal ration	7.14	5.87
Average:		
Basal ration	6.79	6.51
Asparagin	6.64	6.13
Ammonium acetate	6.63	6.16

The experiments of Tryniszewsky also showed substantially the same result, the crude protein of the feces being in period 2, basal ration, 206 grams; period 3, asparagin, 210 grams; period 4, basal ration, 191 grams.

Neither of the foregoing experiments shows very distinct evidence of any increase of the nitrogenous matter of the feces as a result of feeding asparagin or ammonium acetate. It must be remembered, however, that the feces contain nonprotein nitrogen in the form of metabolic products. It is possible, therefore, that the protein nitrogen might have increased in these experiments even though the total nitrogen did not. In more recent experiments, therefore, the comparison has been made upon the protein nitrogen of the feces—that is, the nitrogen which is either insoluble in water or precipitable by copper hydrate. Experiments by Andrlik, Velich, and Stanek,[1] in which glutaminic and aspartic acids were added to the basal ration of a young wether, yielded the following results, which fail to show any material influence of the added nonprotein upon the fecal nitrogen. An earlier series[2] likewise showed no increase in the total nitrogen of the feces as a result of adding betain to the basal ration.

Results of Andrlik, Velich, and Stanek's experiments.

Items.	Feed.		Feces.	
	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.
	Grams.	*Grams.*	*Grams.*	*Grams.*
I. Basal ration	8. 42	1. 190	4. 067	. 390
II. Glutaminic acid	8. 52	3. 072	4. 443	. 450
III. Basal ration	8. 63	1. 190	4. 350	. 350
IV. Aspartic acid	8. 63	3. 296	3. 990	. 410
V. Basal ration	8. 58	1. 190	4. 425	. 350
Average on basal ration	8. 39	1. 190	4. 281	. 363
Average on nonprotein ration	8. 58	3. 184	4. 216	. 430

The experiments upon which special stress has been laid, however, are those of Völtz[3] and of Friedländer.[4]

Völtz fed to a sheep a ration consisting of straw, potatoes, and molasses, or the distiller's residues from the latter—that is, a ration poor in protein and rich in nonprotein. The results, so far as they bear upon the particular point under discussion, are shown in the following table, from which it appears that without exception more protein was found in the feces than in the feed.

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[1] Andrlik, K., and Velich, K. Ueber die bedeutung der Glutamin und Asparaginsäure als Nahrstoffe Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 32, Heft 6, pp. 313–342. Prag, 1908.

[2] Velich, Alois, and Stanek, Vladimir. Ueber das Betain in physiologischchemischer Beziehung. Zweiter Bericht. Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 29, Heft 4, pp. 205–219. Prag, 1905.

[3] Völtz, W. Üeber die Verwertung des Amidgemisches der Melasse durch den Widerkäuer. Archiv für gesammte Physiologie, Band 117, Heft 10–12, pp. 541–563. Bonn, 1907.

[4] Friedländer, Konrad. Zur Frage des Elweissersatzes durch Amide. Die Landwirtschaftlichen Versuchs-Stationen, Band 67, pp. 283–312. Berlin, 1907.

Protein nitrogen in feed and feces—Völtz's experiments.

Periods.	Daily feed.				Protein nitrogen per day.	
	Straw.	Potatoes.	Molasses.	Distiller's residue from molasses.	In feed.	In feces.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
I	500.0	500	....	200	3.42	4.41
II	498.4	500	400	....	3.42	3.92
III	497.9	500	400	....	3.42	3.43
IV	498.4	....	600	....	2.96	3.61
V	394.0	....	560	....	2.42	2.98

In Friedländer's experiments also two sheep received rations poor in protein but containing considerable nonprotein in the form of beet molasses. In two periods asparagin was added to this ration and in one a form of commercial protein. The results are shown in the following table, from which it appears that in this case, too, the protein nitrogen of the feces was in excess of that in the feed, except in the period in which commercial protein was fed.

Protein nitrogen in feed and feces—Friedländer's experiments.

Periods.	Rations.				Protein nitrogen.			
	Hay.	Molasses-peat.	Asparagin.	Protein.	Sheep I.		Sheep II.	
					In feed.	In feces.	In feed.	In feces.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
I	200	625	....	....	3.32	4.01	3.32	4.09
II	200	730	....	....	3.45	5.56	3.43	5.34
III	200	730	30	....	3.16	4.84	....	....
IV	200	625	....	52	10.56	4.35	10.56	4.21
V	200	625	30	....	3.32	4.44	3.32	4.18

Both these experiments have been interpreted as showing a formation of indigestible protein from the nonprotein of the feed.

Just[1] experimented upon two growing lambs through 10 periods. In the first and last periods a basal ration consisting of hay, starch, and sugar was fed. In the intermediate periods nitrogenous matter was added in various forms, while an attempt was made to keep the so-called starch values of the rations unchanged by diminishing the starch and sugar, although this object was not entirely attained. In the following table is shown for each period the difference as regards nonprotein nitrogen and protein nitrogen between the feed and the feces of the period and the average of the two basal rations.

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[1] Just, Jaroslav. Vergleichende Untersuchungen über die Wirkungen des Eiweisses und einiger nichteiweissartiger Stickstoffverbindungen auf den Fleischansatz beim Wiederkäuer. Die landwirtschaftlichen Versuch-Stationen, Band 69, pp. 393–460. Berlin, 1908.

Protein and nonprotein nitrogen in feed and feces—Just's experiments.

	Feed.		Feces—Lamb I.		Feces—Lamb II.	
	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Period 2 (molasses)	2.62	10.61	0.95	7.00	1.22	7.12
Basal	1.36	7.95	.76	5.94	0.75	5.88
	1.26	2.66	.19	1.06	.47	1.24
Period 3 (gluten)	[1] 1.52	[1] 11.17	1.03	6.00	.90	6.21
Basal	1.36	7.95	.76	5.94	.75	5.88
	.16	3.22	.27	.06	.15	.33
Period 4 (malt sprouts extract)	3.53	9.10	1.42	7.07	1.48	6.76
Basal	1.36	7.95	.76	5.94	.75	5.88
	2.17	1.15	.66	1.13	.73	.88
Period 5 (gluten)	1.48	10.51	.89	5.96	.83	5.99
Basal	1.36	7.95	.76	5.94	.75	5.88
	.12	2.56	.13	.02	.08	.11
Period 6 (potato "flocken")	3.24	10.67	1.16	6.11	.97	6.42
Basal	1.36	7.95	.76	5.94	.75	5.88
	1.88	2.72	.40	.17	.22	.54
Period 7 (gluten)	1.53	11.82	.82	6.36	.69	6.13
Basal	1.36	7.95	.76	5.94	.75	5.88
	.17	3.87	.06	.42	−.06	.25
Period 8 (grass extract)	3.35	8.14	.43	6.33	.43	6.35
Basal	1.36	7.95	.76	5.94	.75	5.88
	1.99	.19	−.33	.39	−.32	.47
Period 9 (gluten)	1.51	11.10	.58	6.08	.51	6.32
Basal	1.36	7.95	.76	5.94	.75	5.88
	.15	3.15	−.18	.14	−.24	.44

[1] For Lamb III, 1.49 and 10.77, respectively.

Summarizing these differences, we find that the average increase of protein nitrogen of the feces over that present in the basal periods was as shown in the following table:

Increases in protein nitrogen of feces—Just's experiments.

Items.	Lamb I.	Lamb II.
	Grams.	*Grams.*
Average of gluten	0.16	0.28
Molasses	1.06	1.24
Malt sprouts extract	1.13	.88
Potato "flocken"	.17	.54
Grass extract	.39	.47

While Just's results afford no instance in which the protein nitrogen of the feces exceeds that of the feed, they show a marked effect of the extracts and especially of the molasses in increasing the former.

Kellner and his associates [1] have recently reported the results of two series of experiments upon growing lambs, using a ration composed of oat straw, starch, and sugar; that is, one containing very little protein and practically no nonprotein. To this mixture there was added in the first period ammonium acetate and asparagin and in the second period wheat gluten containing a slightly smaller amount of nitrogen, the energy value of the ration being kept the same by a reduction in the amount of starch. In the second series of experiments, a third period was also added in which ammonium acetate and asparagin were added to the ration of the second period. The results, so far as they relate to the question under discussion, are as shown in the following table.

Although in period 1 of each series the protein nitrogen of the feces is greater than that of the feed, a comparison with period 2 shows that this is not due to any materially greater excretion of protein nitrogen when the ammonium acetate was fed—the amounts being sensibly the same—but to the very small amount of true protein contained in the ration.

Protein and nonprotein nitrogen in feed and feces—Kellner's experiments.

Items.	In feed.		In feces.	
	Nonprotein nitrogen.	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.
	Grams.	*Grams.*	*Grams.*	*Grams.*
Series I:				
Lamb I. Period 1	11.01	1.18	2.53	4.20
Period 2	.92	10.19	.67	4.25
Series II:				
Lamb II. Period 1	14.27	1.55	.71	4.37
Period 2	1.12	11.74	1.44	4.50
Period 3	14.89	11.62	1.03	4.48
Lamb III. Period 1	14.27	1.55	1.76	4.92
Period 2	1.12	11.72	1.86	4.33
Period 3	14.89	11.62	1.24	4.73

The foregoing results make it clear that there is a marked difference between different forms of nonprotein as regards their effect upon the excretion of protein nitrogen in the feces. While Kellner's results (both the earlier and later ones) and those of Tryniszewski show no increase as the result of the addition or substitution of ammonium salts or asparagin, those of Völtz, Friedländer, and Just show a marked increase from the use of plant extracts, especially molasses. Moreover, Just's results show striking differences between the various materials which are by no means related to the content of nonprotein, as is evident from the following table, which shows the increase in the nonprotein nitrogen of the basal ration caused by the addition of the materials named, and the average increase of the protein nitrogen in the feces.

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[1] Kellner, O., Eisenkolbe, P., Flebbe, R., and Neumann, R. Untersuchungen über den Einfluss einiger nicht-eiweissartiger Stickstoffverbindungen auf den Eiweissumsatz beim Wiederkäuer. Die landwirtschaftlichen Versuch-Stationen, Band 72, pp. 437–458. Berlin, 1910.

Comparative increase of nonprotein nitrogen in feed and feces—Just's experiments.

Items.	Nonprotein nitrogen added to basal ration.	Increase of protein nitrogen in feces.
	Grams.	*Grams.*
Gluten	0.17	0.22
Molasses	1.26	1.15
Malt sprouts extract	2.17	1.00
Potato "flocken"	1.88	.36
Grass extract	1.99	.43

These general results are abundantly confirmed by those obtained by Morgen and his associates in the course of their extensive investigations upon the nutritive value of nonproteins for milk production. The results of these experiments will be stated in greater detail immediately in discussing another phase of the subject. In a considerable number of these experiments they consistently observed no increase in the protein nitrogen of the feces to result from the substitution or addition of ammonium salts or asparagin, while, on the other hand, plant extracts had a marked but variable effect in this direction.

These well-established facts are scarcely consistent with the hypothesis of the formation of indigestible bacterial protein from the nonprotein of the feed. If such a formation takes place, it is difficult to see why it should not be quite as marked in the case of readily soluble and assimilable nitrogenous substances like ammonium salts as in that of plant extracts, nor why the effect in the latter case should not be more or less proportional to the amount of nonprotein present.

Moreover, Morgen[1] has shown that plant extracts containing relatively little nonprotein nitrogen may also cause an increase of the protein nitrogen of the feces. He compared extracts prepared from grass and from dried sugar-beet pulp, containing in the dry matter—

	Dried beet-pulp extract.	Grass extract.
	Per cent.	*Per cent.*
Protein	2.84	9.45
Nonprotein	.97	4.20
	3.81	13.65

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[1] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über die Verwertung der Ammonsalze und der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel für die Lebenserhaltung und Milchbildung, sowie über die Frage, ob aus diesen Stoffen unverdauliches Eiweiss gebildet wird. Die landwirtschaftlichen Versuch-Stationen, Band 73, pp. 285-396. Berlin, 1910. See pp. 320 and 350.

In experiments in which these extracts were added to a basal ration, equivalent amounts of starch and sugar were withdrawn, and likewise an amount of protein equal to the true protein of the extracts, disregarding the nonprotein. Upon the average of two animals, the protein and the nonprotein nitrogen of the feed and the protein nitrogen of the feces were as shown in the following table. While the extract of dried sugar-beet pulp increased the nonprotein of the feed somewhat, the increase of protein nitrogen in the feces is relatively much greater than that caused by a much larger increase of nonprotein in the grass-extract ration.

Comparison of extracts of dried beet pulp and of grass—Morgen's experiments.

Items.	In feed.		In feces.
	Protein nitrogen.	Nonprotein nitrogen.	Protein nitrogen.
	Grams.	*Grams.*	*Grams.*
Basal ration	18.32	0.68	5.39
Extract of dried beet pulp	18.61	1.03	6.11
Grass extract	19.35	2.33	7.43

Still further, Morgen[1] found that in numerous digestion experiments upon straw—that is, a feed containing practically no nonprotein—the nitrogen of the feces exceeded that of the feed in 15 cases out of 18. This was by no means a new observation, there being, as Morgen points out, numerous experiments on record showing an apparent negative digestibility of the nitrogen in straw and similar feeding stuffs poor in protein.

The obvious explanation of this phenomenon in the case of the straw is the presence of the so-called nitrogenous metabolic products in the feces. Their presence and their influence upon the apparent digestibility of the food were early recognized, but the first attempt at a quantitative determination of their amount in the excreta of herbivorous animals is due to Kellner,[2] who estimated the average amount of metabolic nitrogen in the feces at 0.4 gram per 100 grams organic matter digested. Subsequent investigations by Pfeiffer[3] and by G. Kühn[4] have resulted in the development of a method by which the amount of metabolic nitrogen contained in the feces can be at least approximately determined. The method is based upon the process of artificial digestion with pepsin or pepsin and trypsin as early proposed by Stöckhardt and by Hofmeister and worked out later by Stutzer [1] for the laboratory determination of the digestibility of feeds. A comparison of Stutzer's method with natural digestion early showed a lower result in the latter case, especially on coarse fodders and those poor in nitrogen; and since it is difficult to see how the action of the digestive juices could be less effective than that of the same enzyms in the laboratory, this led to the conclusion that the difference was due to metabolic nitrogen. To test this, Pfeiffer fed 2 pigs a nitrogen-free ration of starch, sugar, oil, paper pulp, and ash ingredients, to which in a subsequent period pure digestible protein (conglutin) was added. From the first experiment the feces contained an average of 0.4 gram nitrogen per 100 grams of digested dry matter, which must have been in the form of metabolic products, since the feed contained no nitrogen. In the second, in which the protein was assumed to be entirely digestible, practically the same result (0.39 gram metabolic nitrogen) was obtained, showing that the result of the first experiment was not rendered abnormal by the lack of nitrogen in the feed.

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[1] Loc. cit., Band 73, p. 337.

[2] Kellner, O. Beiträge zur quantitativen Bestimmung des verdauten Proteins. Biedermann's Centralblatt für Agrikulturchemie, Jahrgang 9, pp. 107-110. Leipzig, 1880. Untersuchungen über Protein verdanung. Biedermann's Centralblatt für Agrikulturchemie, Jahrgang 9, pp. 763-765. Leipzig, 1880.

[3] Pfeiffer, Th. Beiträge zur Frage über die Bestimmung der Stoffwechselproducte im tierischen Koth. Journal für Landwirtschaft, Jahrgang 33, pp. 149-192. Berlin, 1885.

Pfeiffer, Th. Die Verdaulichkeit getrockneter Rübenschnitzel, sowie die Bestimmung der Verdauungscoëfficienten stickstoffhaltiger Futterbestandtheile im allgemeinen. Journal für Landwirtschaft, Jahrgang 34, pp. 425-453. Berlin, 1886.

Pfeiffer, Th. Die Bestimmung des Stickstoffs der Stoffwechselproducte. Zeitschrift für physiologische Chemie, Band 10, pp. 561-576. Strassburg, 1886.

[4] Kühn, Gustav, Thomas, A., Böttcher, O., Köhler, A., Zielstorff, W., and Barnstein, A. Untersuchungen über die Verdauung stickstoffhaltiger Futterbestandteile durch Behandlung mit Magen- und Pankreas-Extrakten. Die landwirtschaftlichen Versuchs-Stationen, Band, 44. pp. 188-256. Berlin, 1894

For the present purpose, the most interesting feature of the investigation is that these metabolic nitrogenous materials in the fresh dung are soluble in pepsin hydrochloric acid, and can thus be removed from the feces, leaving a residue insoluble in pepsin which represents substantially the indigestible nitrogenous matter of the feed. Kellner [2] also reports similar unpublished results upon a sheep receiving non-nitrogenous matter.[3] That this is actually the case is shown by comparisons of the pepsin-insoluble nitrogen in the feces with that contained in the feed, such as have been made by Pfeiffer and by Kühn. The latter in particular has shown that by certain modifications of Stutzer's method very close agreement can be obtained between artificial and natural digestion if the comparison in the latter case be made upon the pepsin-insoluble nitrogen of the feces. In other words, the pepsin-insoluble nitrogen of the feed reappears quantitatively in the feces.

If, however, indigestible bacterial protein is formed from the nonprotein of the feed, this process should increase the pepsin-insoluble nitrogen of the feces, while if the observed increase in the fecal nitrogen in some cases is due to metabolic products these should be soluble in pepsin. That is, we may regard the pepsin-insoluble nitrogen of the feces as representing indigestible feed protein, and the pepsin-soluble nitrogen as contained in metabolic products, part of which are protein (mucus, epithelium, etc.) and part nonprotein (residues of digestive fluids, etc.). A comparison of the pepsin-insoluble nitrogen in the feed and feces, therefore, affords the best available means of determining whether the ingestion of nonprotein has resulted in the formation of indigestible bacterial protein. Such comparisons have been made in the investigations by Morgen et al., already referred to.

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[1] Stutzer, A. Die Einwirkung von saurem Magensaft auf die stickstoffhaltigan Bestandtheile der Mohnkuchen. Journal für Landwirtschaft, Jahrgang 28, pp. 195–208. Berlin, 1881.

Stutzer, A. Beiträge zur Werthbestimmung der Futtermittel. Journal für Landwirtschaft, Jahrgang 28, pp. 435–453. Berlin, 1881.

[2] Die Ernährung der Landwirtschaftliche Nutztiere, 5th ed., p. 32.

[3] It is, of course, conceivable that the feces may contain food nitrogen which was potentially soluble but which has escaped solution, but it hardly seems likely that any such nitrogen would be dissolved by further treatment with pepsin in the laboratory.

The results of the experiments in 1907 [1] include digestion experiments on 3 ewes in milk and a partial report of experiments upon 2 milch goats. The basis of the ration consisted of dried sugar beet pulp, straw, straw pulp, and a small amount of hay, and contained very little nonprotein. To this basal ration there were added wheat gluten, starch, oil, and sugar. In the experiments upon sheep a portion of the protein supplied by the wheat gluten was replaced in certain periods by nonprotein contained in an extract prepared from malt sprouts. Owing to the rather unpalatable nature of the rations, only about three-fourths of the ration of the protein periods was consumed in the nonprotein periods. In the experiments with goats the malt sprouts extract seems to have been simply added to the basal ration. The results of these experiments, so far as they relate to the question under discussion, are contained in the following table:

Forms of nitrogen in feed and feces—Morgen's experiments of 1907.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.
	Grams.	*Grams.*		*Grams.*	*Grams.*	*Grams.*
Sheep 13:						
Period 1, protein	0.81	21.46		0.96	5.24	3.50
Period 2, nonprotein	5.60	11.14		2.47	5.36	3.39
Period 2-period 1	+4.79	−10.32		+1.51	+ .12	− .11
Sheep 25:						
Period 1, protein	.69	17.55		1.18	3.95	3.23
Period 3, nonprotein	3.58	10.21		1.77	3.86	3.07
Period 3-period 1	+2.89	− 7.34		+ .59	− .09	− .16
Sheep 30:						
Period 1, protein	.69	17.55		.93	4.78	2.93
Period 3, nonprotein	1.30	9.15		1.13	2.84	2.87
Period 3-period 1	+ .61	− 8.40		+ .20	−1.94	− .06
Goat 28:						
Periods 1 and 5, basal	.48	11.70		1.43	3.26	2.45
Period 2, nonprotein	5.97	12.05		2.94	5.09	3.96
Period 2-periods 1 and 5	5.49	.35		1.51	1.83	1.51

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[1] Morgen, A., Beger, C., and Westhausser, F. Weitere Untersuchungen über den Einfluss der nichteiweissartigen Stickstoffverbindungen der Futtermittel auf die Milchproduktion. Die landwirtschaftlichen Versuchs-Stationen, Band 68, pp. 333–432. Berlin, 1908.

Forms of nitrogen in feed and feces—Morgen's experiments of 1907—Continued.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.	Nonprotein nitrogen.	Pepsin-soluble protein nitrogen.	Pepsin-insoluble nitrogen.
Goat 28—Continued.	*Grams.*	*Grams.*		*Grams.*	*Grams.*	*Grams.*
Periods 1 and 5, basal	0.48	11.70		1.43	3.26	2.45
Period 4b, protein	.55	17.39		1.96	3.03	3.38
Period 4b-periods 1 and 5	.07	5.69		.53	— .23	.93
Goat 39:						
Period 1, basal	.40	9.50		1.65	2.11	2.41
Period 3a, nonprotein	5.96	9.86		2.34	4.02	3.26
Period 3a-period 1	5.56	.36		.69	1.91	.85

It appears from this table that with the goats the increase of the nonprotein nitrogen caused a distinct increase in the fecal nitrogen, and that of this increase one-fourth to one-third was insoluble in pepsin. Since, however, the pepsin-insoluble nitrogen of the feed was not determined, it is possible that part of this increase may have arisen from a greater amount of the latter in the feed; but obviously only a small part of the increase in the feces can thus be accounted for, since the entire increase of the protein nitrogen of the feed was only 0.36 gram, as compared with 0.85 gram and 1.51 grams in the feces. On the other hand, however, it is to be noted that there appears to have been a similar increase of the pepsin-insoluble nitrogen in Period IVb, in which wheat gluten instead of malt sprouts extract was added to the basal ration.

In the experiments with sheep the difference in the total amount of the ration noted above gives rise to some difficulty in interpreting the results. It appears clear from the table, however, that the pepsin-insoluble nitrogen of the feces was practically unaffected by the substitution of nonprotein for protein.

In the investigations of 1908,[1] digestion experiments were made on 7 ewes in milk, on 2 milking goats, and on 6 wethers, the pepsin-insoluble nitrogen of the feeding stuffs being determined. The experiments on the milk animals were of the same general nature as those of 1907 and the results were similar. Relatively more hay was fed than in the previous year, making the rations more palatable, and they were eaten without residue throughout. A considerable variety of nonprotein-containing materials were used, viz, extracts of malts prouts, grass, and mangels; ammonium acetate, tartarate, and phosphate, and asparagin. Each nonprotein period was interpolated between two protein periods, the nonprotein (or in two cases carbohydrates) being substituted for protein. The following table gives the average results obtained for each form of nonprotein compared with the average of the results with the same animals for the protein rations.

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[1] Morgen, A., Berger, C., and Westhausser, F. Weitere Untersuchungen über die Verwertung der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel sowie der Ammonsalze durch das milchgebende Tier, unter besonderer Berücksichtigung der stickstoffhaltigen Stoffwechselprodukte. Die landwirtschaftlichen Versuch-Stationen, Band 71, pp. 1-170. Berlin, 1909.

Forms of nitrogen in feed and feces—Morgen's experiments of 1908.

Items.	In feed.			In feces.		
	Non-protein nitrogen.	Protein nitrogen.		Non-protein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
Ammonium salts:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Protein rations (16 periods)	0.58	14.77	2.55	0.77	3.17	2.91
Ammonium rations (9 periods)	6.23	8.93	2.33	.86	2.82	2.78
	+5.65	− 5.84	− .22	+ .09	− .35	− .13
Asparagin:						
Protein rations (6 periods)	.56	14.26	2.48	.73	3.18	2.84
Asparagin rations (2 periods)	6.19	8.41	2.25	.91	2.57	2.56
	+5.63	− 5.85	− .23	+ .18	− .61	− .28
Carbohydrates:						
Protein rations (4 periods)	.60	15.45	2.66	.92	2.91	2.76
Carbohydrate rations (2 periods)	.51	9.32	2.48	.65	3.41	2.73
	− .09	− 6.13	− .18	− .27	+ .50	− .03
Malt sprouts extract:						
Protein rations (4 periods)	.64	16.55	2.86	.68	3.31	3.11
Extract rations (2 periods)	7.98	10.91	3.52	2.35	5.82	4.69
	+7.34	− 5.64	+ .66	+1.67	+2.51	+1.58
Grass extract:						
Protein rations (3 periods)	.59	15.10	2.60	.84	3.29	2.97
Extract rations (1 period)	4.32	11.14	3.63	1.03	4.26	5.59
	+3.73	− 3.96	+1.03	+ .19	+ .97	+2.62
Mangel extract:						
Protein rations (2 periods)	.56	14.26	2.48	.84	2.63	2.84
Extract rations (1 period)	4.13	11.08	3.42	1.05	3.24	4.38
	+3.57	− 3.18	+ .94	+ .21	+ .61	+1.54

The experiments with ammonium salts and asparagin and also the substitution of carbohydrates for protein resulted in a very slight decrease of the pepsin-insoluble nitrogen in the feces, which was closely paralleled by a similar decrease in the feed. In other words, these nonprotein materials showed practically no effect upon the fecal nitrogen. The extract experiments, on the other hand, as in the previous year's tests, showed a marked increase in the pepsin-insoluble nitrogen of the feces, of which increase, however, from 40 to 60 per cent is accounted for by the increase in the pepsin-insoluble nitrogen of the feed. The extracts appear, therefore, to have had a distinct effect in increasing the pepsin-insoluble nitrogen of the feces.

This increase, however, is much less than that of the total protein nitrogen, especially in the case of the malt sprouts extract.

The digestion experiments on the wethers in this year were intended to test the question whether the low apparent digestibility of the protein of the malt sprouts extract as computed from the foregoing digestion experiments was due to the formation of indigestible bacterial protein, or to the so-called depression of digestion due to the small amount of protein in the rations. To test this question the animals received in the first period only straw and malt sprouts extract, while in period 2 wheat gluten was added to this mixture. The average result for the four animals was as follows:

Forms of nitrogen in feed and feces—Morgen's experiments of 1908.

Items.	In feed.			In feces.		
	Non-protein nitrogen.	Protein nitrogen.		Non-protein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Period 1, straw and extract	4.68	6.28	1.93	1.33	2.98	2.99
Period 2, same plus gluten	4.73	11.05	2.20	1.55	2.85	2.91
Difference	−.05	−4.77	−.27	−.22	+.13	+.08

As compared with what may be assumed to be the normal results in period 2, the straw and extract ration of period 1, containing much less protein, showed nevertheless on the average a small increase in the protein nitrogen of the feces, which, however, was large enough to be significant in only two out of the four animals (sheep F and G). This increase in the pepsin-soluble portion of the fecal nitrogen may be taken to indicate that some of the food protein escaped digestion; that is, we have here the so-called depression of digestibility due to a relative excess of nonnitrogenous nutrients. The results computed in this way correspond with the digestion coefficients as computed by Morgen.

In a third period with two of the wethers the malt sprouts extract was replaced by starch, sugar, and gluten, the amount of the latter being made approximately equal to the true protein of the malt extract; while in a fourth period, with only one animal, wheat gluten equivalent to the total nitrogen of the malt sprouts extract was given. The results were as follows:

Forms of nitrogen in feed and feces—Morgen's experiments of 1908.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble
Sheep C:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Period 1	4.80	6.60	2.18	1.24	2.63	3.41
Period 3	.05	6.29	1.74	.34	1.81	2.38
Period 1-period 3	4.75	.31	.44	.90	.82	1.03
Sheep F:						
Period 1	4.80	6.21	1.45	1.05	2.79	2.68
Period 3	.05	5.51	1.34	.42	1.49	1.65
Period 1-period 3	4.75	.64	.11	.63	1.30	1.03
Sheep T:						
Period 1	4.80	6.21	1.45	1.05	2.79	2.68
Period 4	.13	10.31	1.53	.22	1.36	2.12
Period 1-period 4	4.67	−4.10	−.08	.83	1.43	.56

We see here the same effect as before of the malt sprouts extract in increasing the total protein nitrogen of the feces and likewise to a less extent the pepsin-insoluble portion of it.

The experiments upon milking animals in 1909[1] were made chiefly with ammonium acetate, which was both substituted for protein in the basal ration and also added to the latter. Similar addition experiments were also made with grass extract and with the extract of dried sugar-beet pulp containing relatively much less nonprotein. The results upon the different animals were, on the whole, very uniform, and the averages of the following table appear sufficient to demonstrate the teaching of the experiments:

Forms of nitrogen in feed and feces—Morgen's experiments of 1909.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
Ammonium acetate added to basal ration (7 trials):	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Acetate ration	10.73	15.72	2.93	1.10	3.16	2.81
Basal ration	.69	15.72	2.93	1.06	3.24	2.83
Difference	10.04	.00	.00	.04	−.08	−.02

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[1] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über die Verwertung der Ammonsalze und der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel für die Lebenserhaltung und Milchbildung, sowie über die Frage, ob aus diesen Stoffen unverdauliches Eiweiss gebildet wird. Die landwirtschaftlichen Versuchs-Stationen, Band 73, Heft 4-5, pp. 285-396. Berlin, 1910.

Forms of nitrogen in feed and feces—Morgen's experiments of 1909—Continued.

Items.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
Ammonium acetate substituted in basal ration (4 trials):	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Acetate ration	11.00	6.14	2.58	1.15	3.08	2.88
Basal ration	.73	16.41	3.06	1.06	3.23	2.96
Difference	10.27	−10.27	−.48	.09	−.15	−.08
Grass extract added to basal ration (2 trials):						
Extract ration	2.33	15.82	3.53	1.09	3.25	4.18
Basal ration	.68	15.44	2.88	.61	2.81	2.59
Difference	1.65	.38	.65	.48	.44	1.59
Dried beet-pulp extract added to basal ration (2 trials):						
Extract ration	1.03	15.38	3.23	.95	2.62	3.49
Basal ration	.68	15.44	2.88	.61	2.81	2.59
Difference	.35	−.06	.35	.34	−.19	.90

It is clear that the ammonium salts produced practically no effect upon the amount of fecal nitrogen excreted in any form. The grass extract, on the other hand, shows the same effect as in previous experiments, viz, an increase of both the protein nitrogen and the pepsin-insoluble nitrogen of the feces, the latter being greater than can be accounted for by the corresponding increase in the feed. On the other hand, as already noted, the beet-pulp extract, containing much less nonprotein nitrogen, produced a relatively greater effect in this direction, particularly as regards the pepsin-insoluble nitrogen, indicating that the result is not caused by the nonprotein.

The digestion experiments of 1909 were made upon 4 wethers with the same general plan and object as in 1908, viz, to test the hypothesis of the formation of indigestible bacterial protein from the nonprotein. In these experiments wheat gluten was added to a basal ration containing little protein and much nonprotein. They differed from the experiments of the previous year in the fact that the nonprotein consisted of ammonium acetate, which, as the results on milking animals just cited show, does not produce indigestible bacterial protein. The basal ration of period 1 consisted of straw and ammonium acetate. The average results for all 4 animals were as shown in the following table:

Forms of nitrogen in feed and feces—Morgen's experiments of 1909.

Period.	In feed.			In feces.		
	Nonprotein nitrogen.	Protein nitrogen.		Nonprotein nitrogen.	Protein nitrogen.	
		Pepsin-soluble.	Pepsin-insoluble.		Pepsin-soluble.	Pepsin-insoluble.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
1	5.54	1.41	1.50	0.26	1.43	1.78
2	5.57	3.02	1.57	.10	1.48	1.78
3	5.66	7.53	1.79	1.17	.58	1.65

As in the previous experiments, the amount of pepsin-insoluble nitrogen in the feces was practically unaffected. On the other hand, the protein nitrogen of the feces as ordinarily computed was much less in amount in period 3, in which the larger quantity of gluten was fed, the total amounts being, respectively, in period 1, 3.21 grams; in period 2, 3.26 grams; in period 3, 2.23 grams.

This on its face would indicate that some of the food protein escaped digestion—that is, that there was a so-called depression of digestibility in periods 1 and 2—but the excessive amount of nonprotein nitrogen contained in the feces in period 3 seems to suggest the possibility of an error in the analysis of the latter.

On the whole, Morgen's results seem to negative the hypothesis of any considerable formation of indigestible bacterial protein from the nonprotein of the feed. As regards ammonium salts and asparagin, they agree in this respect with the investigations already summarized in failing to show any increase of either protein nitrogen or pepsin-insoluble nitrogen in the feces. As regards the extracts of various feeding stuffs, the results also agree with the earlier results in showing an increase of the protein nitrogen, but they also strongly support the conclusion that that increase is largely due to the effect of these extracts in stimulating the formation of metabolic products and in part also to the fact that the extract rations contained more pepsin-insoluble nitrogen relatively than did the rations with which they were compared. It should be added, however, that practically all of Morgen's experiments show a greater increase of pepsin-insoluble nitrogen in the feces than can thus be accounted for, although it is not yet clear what interpretation is to be placed upon this fact.

Finally, it should be noted that these negative results neither prove nor disprove the possibility of a formation of *digestible* protein from the nonprotein of the feed.

NUTRITIVE VALUE OF NONPROTEIN FOR HERBIVORA.

NONPROTEIN A SOURCE OF PROTEIN.

In addition to the earlier investigations of Kellner and of Tryniszewski already referred to, experiments have also been made by Andrlik, Velich, and Stanek.[1] Their preliminary trials showed that betain injected into the blood of a dog reappeared quantitatively in the urine, but that when given by the mouth only about one-third thus reappeared, while in a later experiment of the same sort none was found in the urine. They also failed to find betain in the excreta of a cow consuming considerable amounts of beet molasses, a substance relatively rich in betain.

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[1] Velich, Alois, and Stanek, Vladimir. Ueber das Betain in fysiologischchemischer Beziehung. Zweiter Bericht. Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 29, Heft 4, pp. 205–219. Prag, 1904–1905.

Andrlik, K. and Velich, K. Ueber die Bedeutung der Glutamin- und Asparaginsäure als Nährstoffe. Zeitschrift für Zuckerindustrie in Böhmen, Jahrgang 32, Heft 6, pp. 313–342. Prag. 1907–1908.

After these preliminary trials, an experiment was made on a young wether weighing about 29 kilograms, including trials with betain, glutaminic acid, and aspartic acid. The trials with betain were reported in 1905 and the others in 1908, but all were made in 1904 and apparently constituted a single investigation.

Throughout the experiment the animal received a basal ration of 500 grams hay and 200 grams wheat flour (the latter baked into cakes), with the exception of periods 4 and 5 of the first series, in which one-half of the flour was replaced by starch. This ration approximates to the maintenance requirement of the animal according to the usual standards as regards quantity of feed, but contains considerably more than the minimum of protein. The materials to be tested were simply added to the basal ration.

On the average of all the periods the basal ration contained per day and head:

	Grams.
Total nitrogen	5.05
Protein nitrogen	4.19

In the nonprotein periods the amounts of nitrogen added in the various forms were:

	Grams.
In betain	2.21
In glutaminic acid	1.93
In aspartic acid	2.11

In the first series of trials the periods were short, covering only 5 or 6 days with either one or no intermediate days. In the second series the periods extended over from 14 to 18 days, all of which, however, are included in the averages compared.

Computed in this manner, the gain or loss of nitrogen by the animal was as follows:

Gain or loss of nitrogen—Andrlik, Velich, and Stanek's experiments.

Items.	Gain (+) or loss (−).	Items.	Gain (+) or loss (−).
Series I:	*Grams.*	Series II:	*Grams.*
Period 1, basal ration	+1.14	Period 1, basal ration	+0.97
Period 2, betain	+1.22	Period 2, glutaminic acid	+1.27
Period 3, basal ration	+ .71	Period 3, basal ration	+ .90
Period 4, betain	+ .92	Period 4, aspartic acid	+1.90
Period 5, basal ration	− .42	Period 5, basal ration	+ .97

Omitting periods 4 and 5 of Series I, in which the nitrogen content of the basal ration was reduced, we may compute the following averages apparently showing that the nitrogenous substances added were at least partially utilized in the body of the animal:

	Gram.
For the basal ration	+0.90
For the nonprotein ration	+1.46

The principal evidence regarding the nutritive value of nonprotein for herbivora, however, is derived from later experiments by Kellner on lambs and cows, from the investigations by Morgen et al., whose results as regards the behavior of nonprotein in the digestive tract have just been discussed, and from experiments by the Laboratory for Agricultural Research in Copenhagen.

Kellner[1] experimented on lambs in the belief that growing animals would furnish the most favorable conditions for the utilization of nonprotein. After a vain attempt to use rations containing ammonium acetate or asparagin as the sole source of nitrogen, he succeeded in finding three animals which consumed sufficient amounts of a ration consisting of starch, sugar, ash, asparagin, and ammonium acetate, together with 300 to 400 grams of straw, to enable the experiment to be carried through. This ration contained, of course, a minimum of digestible protein. In a second period the ammonium acetate and asparagin were replaced by wheat gluten nearly equivalent in nitrogen content, and in the case of two of the animals a third period was employed in which ammonium acetate and asparagin equal to that consumed in the first period were added to the ration of the second period. The energy content of the rations was maintained constant by varying the amounts of starch and sugar.

Regarding the pepsin-soluble nitrogen of the straw as the measure of its digestible protein, and counting all the small amount of nitrogenous matter in the starch as digestible, the total content of protein of the basal rations was far below the maintenance requirement of 0.4 kilogram protein per 1,000 kilograms live weight, as appears from the following table:[1]

Digestible protein in basal rations—Kellner's experiments.

Series and periods.	Per head.	Per 1,000 kilograms live weight.
	Grams.	*Kilos.*
Series I:		
Period 1	4.76	0.11
Period 2	4.44	.10
Series II:		
Period 1	5.34	.11
Period 2	4.91	.10
Period 3	4.91	.10

These experiments have already been considered in their bearings upon the fate of the nonproteins in the digestive tract (p. 18). The results as to the production of flesh are shown in the following table of the nitrogen balances per day and head:

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[1] Kellner, O., Eisenkolbe, P., Flebbe, R., and Neumann, R. Untersuchungen über den Einfluss einiger nicht-eiweissartiger Stickstoffverbindungen auf den Eiweissumsatz beim Weiderkäuer. Die landwirtschaftlichen Versuchs-Stationem, Band 72, Heft 5–6, pp. 437–458. Berlin, 1910.

[1] The approximate live weights were 44 kilograms in the first series and 48 kilograms in the second series.

Nitrogen balances—Kellner's experiments.

Items.	Period I.		Period II.		Period III.	
	Total nitrogen.	Protein nitrogen.	Total nitrogen.	Protein nitrogen.	Total nitrogen.	Protein nitrogen.
Series I:						
Lamb 1—	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*
In feed	12.19	1.18	11.11	10.19	....	....
In feces	6.73	4.20	4.92	4.25	....	....
In urine	5.37	....	4.26	....	....	....
In total excreta	12.10	....	9.18	....	....	....
Gain	+ .09	....	+1.93	....	....	....
Series II:						
Lamb 2—						
In feed	15.82	1.55	12.86	11.74	26.51	11.62
In feces	5.08	4.37	5.94	4.50	5.51	4.48
In urine	10.16	....	4.28	....	16.17	....
In total excreta	15.24	....	10.22	....	21.68	....
Gain	+ .58	....	+2.64	....	+4.83	....
Lamb 3—						
In feed	15.82	1.55	12.84	11.72	26.51	11.62
In feces	6.68	4.92	6.19	4.33	5.17	4.73
In urine	10.70	....	5.13	....	16.72	....
In total excreta	17.38	....	11.32	....	22.69	....
Gain	−1.56	....	+1.52	....	+3.82	....

Two general conclusions seem to follow from the foregoing results: First, there appears to be clear evidence of a conversion of nonprotein into protein. Although the amount of true protein in the feed in Period I was about one-fourth that required for maintenance, lamb 1 was fully maintained and lamb 2 showed a small gain of nitrogen, while in case of lamb 3 the protein lost from the body plus that supplied in the feed amounts to only 14.66 grams protein per head, equivalent to 0.31 kilogram per 1,000 kilograms live weight, or about three-fourths of the maintenance requirement. Moreover, the nonprotein added in Period III to the ration of Period II caused a notable increase in the storage of protein in the body, either directly or by taking the place of protein previously used for maintenance purposes.

Second, the nonprotein was clearly inferior to protein, for the substitution of the latter for the former in Period II caused a notable gain of protein by the animals in place of approximate maintenance, even although the supply of total nitrogen in the feed was less than in Period I in every case and that of digestible nitrogen less in two cases out of the three.

Kellner's conclusion is that the ammonium salts and asparagin were synthesized by the bacteria of the digestive tract to more complex nitrogenous compounds, possibly even to proteins, and that these compounds were subsequently digested and served in Period I to maintain the protein of the body. The striking difference between the results of Period I and those of Period II he regards as showing that while nonprotein may thus indirectly perform maintenance functions it is incapable of causing actual growth of protein tissue. The increased gain observed in Period III he explains in the manner previously indicated, viz, that the nonprotein was substituted for protein for maintenance purposes.

But while this explanation is consistent with the experimental results, the existence of such a marked distinction between digested feed protein and digested products of bacterial synthesis appears to the writer unlikely. We can scarcely imagine that this synthesis should result in the production of any simpler compounds than the simple amino acids, while it is more likely to extend at least to the formation of polypeptids if not in part to that of proteins. It is well established, however, that substantially these comparatively simple substances constitute the nitrogenous food of the body, and it is not apparent why their origin through bacterial synthesis should render them any less available than similar substances resulting from enzym cleavage of feed protein. While minor differences may exist, it appears more probable that the limit to the nutritive value of nonproteins to the herbivorous animal is set by the amount which the bacteria are able to synthesize rather than by a difference in the value of the products, and this belief seems to be supported by the investigations at the Hohenheim Experiment Station by Morgen et al. upon milk-producing animals (sheep and goats), about to be considered.

The results of Morgen's investigations have already been discussed in their bearing upon the fate of nonprotein in the digestive tract, but the digestion experiments cited for this purpose formed only part of more extensive investigations, including numerous additional animals, in which the yield and composition of the milk produced were determined.

Perhaps the most striking result of these investigations is the demonstration that nonprotein nitrogen in the form of ammonium salts or asparagin is capable of contributing to the production of milk protein. This conclusion is based upon a comparison of the milk protein with the digestible true protein of the feed, the latter being considered equivalent to the protein nitrogen of feed minus protein nitrogen of feces. At first thought it might seem that only the pepsin-insoluble nitrogen of the feces should be considered in this calculation, since there is good reason to believe that the remainder of the fecal nitrogen is present as metabolic products. But while this is probably true, on the other hand these nitrogenous metabolic products, so far as they are protein in nature (mucus, epithelium, etc.), constitute a loss of protein from the body, and therefore should be taken into account in drawing conclusions from the nitrogen balance.

In the experiments of 1907[1] upon malt sprouts extract, the digestion experiments whose results have already been cited (p. 22) included also determinations of the urinary nitrogen and of the nitrogen in the daily growth of wool. Assuming, on the basis of earlier experiments, a maintenance requirement of 0.4 kilogram digestible protein per 1,000 kilograms live weight (an estimate corresponding almost exactly with Katayama's results[2]), they compute the amount of protein available per day and head for milk production in the periods in which nonprotein was partially substituted for protein as follows:

Protein available for milk production—Morgen's experiments of 1907.

Items.	Sheep 13, period 2.	Sheep 25, period 3.	Goat 28, period 2.	Goat 39, period 3a.
	Grams.	*Grams.*	*Grams.*	*Grams.*
Protein nitrogen available	2.39	3.28	3.00	2.58
Estimated for maintenance	2.88	2.37	2.37	2.56
Remainder	− .49	.91	.63	.02
In growth of wool	.79	.64	....	....
Remainder	−1.28	.27	.63	.02
Loss from body	1.02	1.89	1.53	1.39
Available for milk	− .26	2.16	2.16	1.41
Found in milk	2.29	2.70	3.71	2.83
Deficit	2.55	.54	1.55	1.42
Nonprotein nitrogen in feed	5.60	3.58	5.97	5.96
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Utilization of nonprotein	45.54	15.08	25.96	23.83

In every instance the milk contained more protein than was computed to be available from feed and body protein, while in the extreme case (sheep 13) the feed protein was little more than equal to the milk protein plus wool protein, leaving only 0.33 gram protein nitrogen for maintenance. Evidently the nonprotein must have been utilized either for the production of milk protein or for the maintenance of the body tissues. In the comparison periods in which protein instead of nonprotein was fed, on the other hand, the amount of protein available for milk production, computed in the same manner, was considerably in excess of the protein found in the milk. In general, then, the results of this series of experiments correspond with those obtained in Kellner's experiments on lambs.

The experiments of 1908[3] included two trials on malt sprouts extract, one each upon grass extract and mangel extract, eight upon ammonium acetate, one upon ammonium tartrate, and two upon asparagin, in all of which the nitrogen balance was determined.[1] Computed as before, the average results per day and head for each nonprotein material were as follows:

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[1] Morgen, A., Berger, C., and Westhausser, F. Weitere Untersuchungen über den Einfluss der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel auf die Milchproduktion. Die landwirtschaftlichen Versuchs-Stationen, Jahrgang 68, Heft 5–6, pp. 333–432. Berlin, 1908.

[2] Katayama, T. Über das Eiweiss-Minimum für ausgewachsene Hammel. Die landwirtschaftlichen Versuchs-Stationen, Band 69, Heft 5–6, pp. 321–341. Berlin, 1908.

[3] Morgen, A., Beger, C., and Westhausser, F. Weitere Untersuchungen über die Verwertung der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel sowie der Ammonsalze durch das milchgebende Tier unter besonderer Berücksichtigung der stickstoffhaltigen Stoffwechselprodukte. Die landwirtschaftlichen Versuchs-Stationen, Band 71, Heft 1–3, pp. 1–170. Berlin, 1909.

Protein available for milk production—Morgen's experiments of 1908.

Items.	Ammonium salts.	Asparagin.	Extract of malt sprouts.	Extract of grass.	Extract of mangels.
	Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*
Protein nitrogen available	5.69	5.53	3.93	4.92	6.88
Estimated for maintenance	2.57	2.50	3.14	2.62	2.50
Remainder	3.12	3.03	.79	2.30	4.38
In growth of wool	.47	.54	.70	.48	....
Remainder	2.65	2.49	.09	1.82	4.38
Loss from body	.18	– .01	2.28	.75	– .49
Available for milk	2.83	2.48	2.37	2.57	3.89
Found in milk	4.21	3.92	3.96	2.17	3.51
Deficit	1.38	1.44	1.58	....	....
Nonprotein nitrogen in feed	6.23	6.19	7.98	....	....
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Utilization of nonprotein	22.15	23.26	19.80	....	....

All the experiments, except the single trials upon extracts of grass and of mangels, showed a deficit of available protein as compared with the amount found in the milk, while, as in 1907, the comparison periods in which protein was fed showed a surplus. The exceptional results with the two extracts may, perhaps, be ascribed to the fact that in these trials a smaller proportion of the digestible protein was replaced by nonprotein than in the other cases (about 21 per cent as compared with 33 to 38 per cent), although the replacement was carried as far as in the experiments with sheep in 1907.

The experiments of 1909[2] showed even more striking results in the four cases in which about 63 per cent of the digestible true protein was replaced by ammonium acetate. The individual results, per day and head, computed as in the preceding cases, were:

Protein available for milk production—Morgen's experiments of 1909.

Items.	Sheep 48, period 2.	Sheep 49, period 2.	Sheep 50, period 2.	Sheep 56, period 3.
	Grams.	*Grams.*	*Grams.*	*Grams.*
Protein nitrogen available	2.89	2.73	2.70	2.70
Estimated for maintenance and wool	3.55	3.67	3.35	3.33
Remainder	– .66	– .94	– .65	– .63
Loss from body	– .16	.24	– .45	– .27
Available for milk	– .82	– .70	–1.10	– .90
Found in milk	4.15	3.61	3.55	3.19
Total deficit	4.97	4.31	4.65	4.09
Nonprotein nitrogen in feed	11.65	11.65	10.35	10.35
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Utilization of nonprotein	42.7	37.0	44.9	39.5

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[1] The results as regards digestibility are tabulated on page 24.

[2] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über die Verwertung der Ammonsalze und der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel für die Lebenserhaltung und Milchbildung, sowie über die Frage ob aus diesen Stoffen unverdauliches Eiweiss gebildet wird. Die landwirtschaftlichen Versuchs-Stationen, Band 73, Heft 4-5, pp. 285-396. Berlin, 1910.

The only conclusion which can be drawn from these results is that the ammonium acetate served as a source of milk protein. Any error in the estimate of the maintenance requirement is without significance, since, even if we disregard the slight gain of protein by the body in three cases, the total amount of protein contained in the rations (plus that supplied from the body by sheep 49) is notably less than the amount actually found in the milk. The obvious interpretation of these results is that some of the ammonium acetate was synthesized to protein by means of bacteria and subsequently digested.

On the other hand, the seven trials in which ammonium acetate was added to a basal ration containing a moderate amount of digestible protein, instead of being substituted for the latter, yielded no such results, the true protein present being more than sufficient for all purposes. The same thing was likewise true of the trials in which extracts of grass and of dried beet pulp were added to the basal ration.

A similar result has also been reported by Kellner[1] in an experiment on a milch cow. The protein content of the ration was gradually diminished until the excess over the maintenance requirement was practically equal to the protein of the milk, and then a part of the remaining protein was replaced by ammonium acetate and starch, the total ration in both cases being sufficient to cause some gain of body fat. The results as regards nitrogen were as follows:

Protein available for milk production—Kellner's experiments.

Items.	Period VI, with ammonium acetate.	Period VII, without ammonium acetate.
	Grams.	*Grams.*
Protein nitrogen, digested	22.23	53.83
Estimated for maintenance	28.98	28.98
Remainder	— 6.75	24.85
Gain or loss by body	— 1.96	+ 1.86
Available for milk	— 4.79	22.99
In milk	53.37	55.79
Deficit	58.16	32.80
Nonprotein nitrogen in feed	62.03	34.70
	Per cent.	*Per cent.*
Utilization of nonprotein	93.76	94.52

Even in Period VII there appears to have been a slight formation of milk from the nonprotein of the feed, while in Period VI the phenomenon is even more striking than in Morgen's experiments. Kellner, it is true, has subsequently[2] expressed the opinion that his results do not prove such a utilization of nonprotein, since it is uncertain how much of the indigestible protein found in the feces may have been bacterial protein derived from the nonprotein of the feed. As has been shown in the foregoing pages, however, the evidence is strongly against such an action in the digestive tract, and it would seem that Kellner's results must rank with Morgen's as showing a possible utilization of ammonia for the production of milk protein.

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[1] Kellner, Oskar. Die Ernährung der Landwirtschaften Nutztiere, 4th ed., p. 539.

[2] Kellner, Oskar. Ibid., 5th ed., p. 549.

Experiments upon the minimum protein requirements of dairy cows reported from the Laboratory for Agricultural Research in Copenhagen in 1906–7 [1] afford confirmation of Morgen's results. In these experiments the cottonseed cake contained in the initial ration was gradually withdrawn, while the amount of fodder beets was increased so as to maintain unchanged the number of feed units in the ration computed according to Fjord's system. The protein nitrogen of the ration was thus largely reduced while the amount of nonprotein shows but slight differences. The experiments, therefore, show little as to the behavior of nonprotein in the digestive tract or as to its nutritive value as compared with that of protein. Like Morgen's experiments of 1907 and 1908, however, they appear to show that the nonprotein of the rations must have served at least for the maintenance of the protein tissues of the body, while in some instances the assumption of a small formation of milk protein from nonprotein seems necessary. The following table, compiled from the Sixtieth Report, shows the results of those of the experiments in which the smaller amounts of true protein were consumed. On the average of all these trials, the nonprotein nitrogen constituted about 23 per cent of the total nitrogen. The table shows that in general the protein available from the feed, plus that contributed by the body protein, is approximately equal to the milk protein, so that the maintenance function at least must have been supported by the nonprotein.

Results of Copenhagen experiments—Sixtieth Report.

Items.	Protein nitrogen digested.[2]	Gain of nitrogen by body.	Protein nitrogen available for milk production.	Nitrogen in milk.	Surplus for maintenance.	Estimated maintenance requirement.	Deficit of protein nitrogen.	Nonprotein nitrogen in feed.	Percentage utilization of nonprotein nitrogen.
Cow No. 10:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Per ct.*	*Per ct.*
Period 4	47	−16	63	63	0	32	32	35	91.4
Period 5	44	−12	56	60	−4	32	36	36	100.0
Period 6	57	− 2	59	62	−3	32	35	39	89.7
Cow No. 23:									
Period 4	47	− 9	56	59	−3	35	38	34	111.8
Period 5	47	−15	62	57	5	35	30	36	83.3

[1] Denmark—Beretning fra den Kgl. Veterinaer of Landbohojskoles Laboratorium for landokonomiske Forsog. 60de, 1906, and 63de, 1907, Kobenhavn. Translated by Mallevre, Société de l'Alimentation Rationale du Bétail. Compte Rendu de 11ème et 12ème Congrès.

[2] Protein nitrogen of feed minus protein nitrogen of feces.

Results of Copenhagen experiments—Sixtieth Report—Continued.

Items.	Protein nitrogen digested.	Gain of nitrogen by body.	Protein nitrogen available for milk production.	Nitrogen in milk.	Surplus for maintenance.	Estimated maintenance requirement.	Deficit of protein nitrogen.	Nonprotein nitrogen in feed.	Percentage utilization of nonprotein nitrogen.
Cow No. 53:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Per ct.*	*Per ct.*
Period 4	53	− 2	55	56	−1	30	31	34	91.2
Period 5	28	−13	41	45	−4	30	34	39	87.2
Period 6	50	+ 3	47	49	−2	30	32	41	78.1
Cow No. 68:									
Period 4	57	− 5	62	63	−1	32	33	35	94.3
Period 5	44	−16	60	58	2	32	30	36	83.3
Period 14	48	− 2	50	50	0	32	32	33	97.0
Cow No. 58:									
Period 4	51	− 1	52	52	0	32	32	34	94.1
Period 5	27	−13	40	45	−5	32	37	37	100.0
Period 6	55	+ 6	49	47	2	32	30	38	78.9
Total	655	−97	752	766	−14	448	462	507	91.1

In the experiments recorded in the Sixty-third Report the fact is taken into account that a portion of the nitrogen of the beets existed in the form of nitrates, which, as is well known, yield free nitrogen in the digestive tract. For this reason the loss of protein from the body is somewhat greater than that which would be computed from the income and outgo of nitrogen in the usual way. When the results of the experiments are computed after the ordinary method, they show in several cases a distinct deficit of protein; that is, the protein available from the food plus that contributed by the body is distinctly less than the protein of the milk. When the correction for nitrates is applied, however, these differences either disappear or become almost negligible, as is shown by the following table:

Results of Copenhagen experiments—Sixty-third Report.

Items.	Protein nitrogen digested.	Gain of nitrogen by body.	Protein nitrogen available for milk production.	Nitrogen in milk.	Surplus for maintenance.	Estimated maintenance requirement.	Deficit of protein nitrogen.	Nonprotein nitrogen in feed.	Percentage utilization of nonprotein nitrogen.
Cow No. 68:	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Per ct.*
Period 3	57	−16	73	65	8	30	22	35	62.9
Period 4	33	−26	59	59	....	30	30	35	85.7
Period 5	30	−23	53	58	−5	30	35	36	97.2
Period 6	53	− 4	57	59	−2	30	32	39	82.1
Cow No. 125:									
Period 4	69	−17	86	83	3	33	30	55	54.6
Period 5	69	−12	81	83	−2	33	35	54	64.8
Period 6	87	+ 4	83	79	4	33	29	55	52.7
Period 7	94	+ 7	87	79	8	33	25	54	46.3

EFFECT OF NONPROTEIN ON TOTAL PRODUCTION.

But while Morgen's results on milking animals differ from Kellner's on lambs in this one point, their results are entirely in accord in showing that the nutritive value of nonprotein is much inferior to that of protein. This becomes apparent as soon as we turn from a study of the digestion trials and nitrogen balances to a consideration of the actual yield of milk and its constituents on the various rations. The preliminary experiments of 1906[1] showed a marked decrease in the milk production when nonprotein was substituted for protein, and the more elaborate experiments of the following years only confirmed this result.

The experiments were made after the so-called "period system," the natural decrease in the milk yield with advancing lactation being estimated by a comparison between an initial and a final period on identical rations. In comparing his results Morgen simply adds the correction thus computed for each period to the observed yield, and thus computes what the yield would have been had there been no depression due to advancing lactation, and these corrected numbers constitute the basis for comparing the effects of the rations. It may easily be shown, however, that this method of computation is incorrect and tends to reduce the real effects of the changes in the rations. For example, in one experiment the average daily yields of milk solids were:

	Grams.
Period 1, protein ration	105.70
Period 2, malt sprouts extract ration	36.62
Period 3, protein ration	36.25

From the middle of period 1 to the middle of period 3 was 78 days, so that the average daily falling off in the yield of milk solids was 0.8904 gram, and for the 40.5 days between period 1 and period 2 amounted to 36.08 grams. Morgen therefore makes the following comparison of the yields of milk solids:

	Grams.	Grams.
Period 1		105.70
Period 2:		
Observed	36.62	
Correction	36.08	
Computed		72.70
Period 3:		
Observed	36.25	
Correction	69.45	
Computed		105.70

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[1] Morgen, A., Beger, C., and Westhausser, F. Untersuchungen über den Einfluss der nicht-eiweissartigen Stickstoffverbindungen der Futtermittel auf die Milchproduktion. Die landwirtschaftlichen Versuchs-Stationen, Band 65, Heft 5-6, pp. 313-440. Berlin, 1906-7.

and computes that the effect of the extract ration was 72.70 ÷ 105.70 = 68.8 per cent of that of the protein ration. The assumption underlying such a correction for the advance of lactation, however, is that if the feed had been unchanged the falling off in yield would have been proportional to the time. In this case, therefore, the falling off up to the middle of period 2 would have been, as computed, 36.08 grams, and consequently the yield of milk solids on an unchanged ration would have been 105.70 − 36.08 = 69.62 grams, so that we may make the following comparison:

Periods.	Observed yield.	Computed yield.	Observed in percentage of computed.
	Grams.	*Grams.*	*Per cent.*
1	105.70		
2	36.62	69.62	52.61
3	36.25		

Morgen's method of computation, in other words, adds the same correction to two unequal quantities; and therefore, while the difference between the two is unaffected, the ratio between them is distorted in favor of the smaller number.

In the experiments of 1907 the basal rations contained approximately 2.5 kilograms of digestible protein per 1,000 kilograms live weight, of which approximately 0.9 kilogram was replaced by the nonprotein of malt sprouts extract. The following table shows the production of milk solids and of milk protein expressed as a percentage of the amount which it is computed would have been produced had the protein ration been continued unchanged, the computation being made in the manner just indicated.[1]

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[1] In certain of the experiments only a three-fourths ration could be fed in the nonprotein periods, and corresponding periods were introduced in which three-fourths of the normal protein ration was fed. In these cases the results have been computed on the assumption that the rate of decrease in milk production would have been the same as was actually observed between the two full protein rations. For example, in case of sheep 13 (loc. cit., p. 402) the following results were obtained:

	Milk solids.	Milk protein.
	Per cent.	*Per cent.*
Correction (period 3-2)	22.85	1.02
Observed yield in period 3 on three-fourths protein ration	46.47	2.54
Computed yield in period 2 on three-fourths protein ration	69.32	3.56
Observed yield in period 2	40.78	2.29
Observed in percentage of computed	58.84	64.33

Yield in per cent of yield in protein periods—Morgen's experiments of 1907.

Items.	Milk solids.	Milk protein.
Malt sprouts extract periods:	*Per cent.*	*Per cent.*
Sheep 13	58.84	64.33
Sheep 22	84.38	83.58
Sheep 25	75.38	74.62
Sheep 27	58.07	54.72
Sheep 27 (grass extract)	75.48	65.57
Sheep 30	71.52	71.16
Sheep 32	69.00	67.76
Sheep 34	61.44	59.76
Average of percentages	69.28	67.69
Carbohydrate periods:		
Sheep 22	74.49	73.71
Sheep 27	59.12	55.56
Sheep 30	83.58	83.59
Sheep 32	61.62	57.99
Sheep 34	52.12	52.49
Average of percentages	66.19	64.67

In general, the relative yield in the malt sprouts extract periods is somewhat greater than that obtained from a corresponding quantity of carbohydrates, although there are individual exceptions. Apparently the nonprotein, while greatly inferior to protein, had a somewhat greater nutritive value than the carbohydrates.

In the experiments of 1908 the basal ration contained 2.3 kilograms digestible protein per thousand live weight. In the various periods either 28 per cent or 44 per cent of this protein was replaced by nonprotein derived from various sources, viz, from malt sprouts extract, grass extract, mangel extract, ammonium salts, and asparagin, while six carbohydrate periods were also introduced. The relative yields, computed as in the preceding case, using the yield in periods 1 and 5 on the protein ration as the basis of computation, were as follows:

Yield in per cent of yield in protein periods—Morgen's experiments of 1908.

Items.	Percentage of protein replaced.	Milk solids.	Milk protein.
Malt sprouts extract:		*Per cent.*	*Per cent.*
Sheep 13	44	52.61	62.40
Sheep 22	44	79.67	79.97
Sheep 27	28	67.44	70.55
Sheep 30	44	55.91	55.56
Sheep 32	44	63.71	64.63
Sheep 33	44	48.57	48.70
Sheep 42	28	51.92	52.74
Goat 53	28	82.26	85.71
Average of percentages	....	62.76	65.03
Grass extract:			
Sheep 49	28	53.08	46.77
Goat 31	28	84.39	82.87
Goat 38	28	73.72	62.86
Goat 41	28	54.51	48.28
Goat 45	28	62.92	53.62
Goat 52	28	69.05	57.49
Goat 53	28	85.71	89.18
Average of percentages	....	69.05	63.01

Yield in per cent of yield in protein period—Morgen's experiments of 1908—Continued.

Items.	Percentage of protein replaced.	Milk solids.	Milk proteins.
		Per cent.	*Per cent.*
Mangel extract:			
Sheep 34	28	72.96	68.21
Sheep 42	28	59.08	51.74
Goat 28	28	89.96	84.79
Average of percentages	....	74.00	68.25
Ammonium salts:			
Sheep 34, acetate	28	102.35	93.75
Sheep 35, acetate	44	93.11	83.03
Sheep 35, acetate as drink	44	107.30	90.33
Sheep 37, acetate	44	80.01	75.75
Sheep 37, acetate as drink	44	78.12	74.25
Sheep 48, tartrate	44	62.28	66.91
Sheep 48, acetate	44	86.32	79.50
Sheep 49, acetate	44	72.97	72.70
Sheep 50, acetate	44	69.99	73.77
Goat 28, acetate	28	94.69	86.27
Goat 40, phosphate	44	81.13	85.11
Goat 41, acetate	28	87.34	82.96
Goat 45, acetate	28	66.13	70.51
Average of percentages	....	83.21	79.60
Asparagin:			
Sheep 35	44	80.50	81.82
Sheep 50	44	63.44	61.28
Average of percentages	....	71.97	71.55
Average of ammonium salts and asparagin	....	81.72	78.54
Carbohydrates:			
Sheep 30	44	54.53	52.74
Sheep 33	44	56.96	57.33
Average of percentages	....	55.75	55.04
Sheep 31	28	93.63	95.47
Sheep 38	28	83.78	82.06
Sheep 40	44	95.24	94.70
Sheep 52	28	72.65	70.35
Average of percentages	....	86.33	85.65
Protein withdrawn:			
Sheep 32	44	63.26	64.25
Averages:			
Malt sprouts extract	....	62.76	65.03
Grass extract	....	69.05	63.01
Mangel extract	....	74.00	68.25
Ammonium salts and asparagin	....	81.72	78.54
Carbohydrates—			
Sheep	....	55.75	55.04
Goats	....	86.33	85.65

The results upon the individual animals were more or less variable, a fact probably due in part to differences in the order of the various periods and perhaps to individual differences. In general, the extracts gave results fully as low as in the experiments of 1907, but also somewhat higher than those obtained in the carbohydrate periods with sheep. The results with goats in the latter periods seem exceptional. On the other hand, the relative yield upon ammonium salts and asparagin was notably greater than on the plant extracts, although falling materially short of that obtained with protein.

It should be noted that the low value of the extracts as compared with ammonium salts may be due to a small extent to a specific effect of these materials on the milk production, as appears from the results of the next year.

In the experiments of 1909 the basal ration contained 2.4 kilograms of digestible protein per thousand live weight. In eight trials 63 per cent of this was replaced by ammonium acetate containing an equal amount of nitrogen. In the other trials ammonium acetate and extracts of grass, malt sprouts, and dried beet pulp were simply added to the basal ration. The results, computed as before, are contained in the following table:

Yield in per cent of yield in protein periods—Morgen's experiments of 1909.

Items.	Milk solids.	Milk protein.
Ammonium acetate—substitution experiments:	*Per cent.*	*Per cent.*
Sheep 32	52.89	54.63
Sheep 48	60.68	58.13
Sheep 49	57.68	56.94
Sheep 50	64.85	61.42
Sheep 56	58.96	59.30
Sheep 57	73.50	75.26
Sheep 61	59.69	64.63
[Goat 51]	[88.37]	[77.58]
Average of percentages (omitting goat 51)	61.04	61.47
Ammonium acetate—addition experiments:		
Sheep 32	85.49	91.08
Sheep 48	79.44	74.38
Sheep 49	93.57	91.62
Sheep 50	93.06	89.34
Sheep 56	108.44	102.23
Sheep 57 (period 3)	95.39	99.17
Sheep 57 (period 4)	107.55	105.21
Sheep 61	94.29	92.46
[Goat 51]	[144.25]	[142.20]
Average of percentages (omitting goat 51)	94.15	93.19
Grass extract—addition experiments:		
Sheep 35	85.33	82.96
Sheep 42	93.77	85.91
Goat 45	93.57	90.07
Average of percentages	90.89	86.31
Malt sprouts extract—addition experiments:		
Goat 38	93.17	91.25
Goat 40	91.50	85.51
Goat 52	92.46	87.07
Average of percentages	92.38	87.94
Beet-pulp extract—addition experiments:		
Sheep 35	86.87	84.84
Sheep 42	80.91	73.21
Goat 38	91.66	89.45
Goat 40	96.51	86.23
Goat 45 (period 3)	77.60	75.64
Goat 45 (period 4)	77.41	73.55
Goat 52	84.56	83.78
Average of percentages	84.93	80.96
Averages:		
Ammonium acetate—substitution experiments	61.04	61.47
Ammonium acetate—addition experiments	94.15	93.19
Grass extract—addition experiments	90.89	86.31
Malt sprouts extract—addition experiments	92.38	87.94
Beet-pulp extract—addition experiments	84.93	80.96

In the substitution experiments with ammonium acetate, the milk yield fell off much more than in 1908, obviously because the substitution was carried much further. On the other hand, the addition of ammonium salts to the basal ration produced practically no effect upon the yield, the small differences noted being scarcely significant. The addition of the various extracts seems to have caused some decrease in the yield, which, as already suggested, may indicate a specific effect, but one which is not great enough to account for the falling off noted in the substitution experiments of the previous year.

The foregoing results fully confirm the conclusions drawn from a study of the nitrogen balances, and show that the ammonium salts, asparagin, and plant extracts all seem to have enabled the animals to produce a greater amount of milk than did a corresponding amount of carbohydrates. With the plant extracts this difference is comparatively slight, but with ammonium acetate and asparagin it is quite decided. It would appear as if these latter readily soluble materials are more easily converted into protein by the bacteria of the digestive tract than are the diverse nonprotein substances of plant extracts.

On the other hand, the amount of nonprotein nitrogen thus rendered available was relatively small. The organism reacted to the replacement of protein by ammonium salts chiefly by diminishing the amount of milk produced, while under these conditions it was able to utilize that portion of the nonprotein rendered available by bacterial action. When, however, the protein supply was reasonably abundant, the amount which may have been formed from the added nonprotein under those circumstances produced no perceptible effect upon the milk yield.

These relations are perhaps most apparent in the case of the four digestion experiments of 1909 in which the protein of the basal ration was replaced by ammonium acetate (p. 34). If we compute in the same manner as in the foregoing cases what the amount of nitrogen in the milk yield would have been had the protein ration been continued, we get the following results:

Influence of feed protein on yield of milk protein—Morgen's experiments of 1909.

Items.	Yield of milk protein.	
	Observed for nonprotein ration.	Computed for protein ration.
Yield of nitrogen in milk:	*Grams.*	*Grams.*
Sheep 48	4.15	7.14
Sheep 49	3.61	6.34
Sheep 50	3.55	5.78
Sheep 56	3.19	5.38
Average	3.63	6.16
Average available protein nitrogen of feed	2.76	13.28

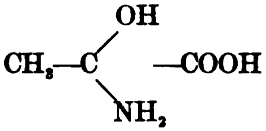
On the protein rations the supply of food protein was sufficient to cover the demand for maintenance, the gain of protein by the body, and the production of protein in the milk, and to leave an average surplus of 3.48 grams nitrogen. When, however, the available protein supply was reduced to the low figure of 2.76 grams nitrogen, or 21 per cent of the previous amount, the yield by the animals was reduced in the case of the milk solids by 39 per cent and in the case of the milk protein by 41 per cent. This large falling off makes it evident that the protein supply was the limiting factor of milk production in these periods. Under these conditions of limited protein supply, however, the nonprotein nitrogen of the feed was utilized to a certain extent as is shown by the nitrogen balances, so that the falling off in the yield of milk was not proportional to the reduction in the protein supply.

DIRECT UTILIZATION OF AMMONIUM SALTS.

It will not have escaped notice that the evidence of the utilization of nonprotein by means of the formation of bacterial protein which is furnished by the foregoing experiments is indirect. Since it has not been satisfactorily shown that carnivora or omnivora can utilize nonprotein as a source of protein, it is concluded that the opposite results with herbivora can not be ascribed to a synthetic production of protein in the processes of metabolism, but must be due to some other cause, the formation of bacterial protein appearing the most probable one.

Moreover, the most decided nutritive effect is obtained with ammonium salts, i. e., precisely those compounds which seem least likely to be subject to metabolic synthesis. In this connection, however, attention should be called to two recent papers.

Knoop[1] claims to have established the theoretical possibility of a formation of amino acids in the body of the dog from ammonia and nonnitrogenous substances. According to him, the deamidization of the amino acids resulting from protein cleavage in metabolism is a process of oxidation, giving rise to the corresponding keto and oxy acids and ammonia. This change he regards as a reversible reaction and assumes the formation of hypothetical intermediate products of the type



$$CH_3-C(OH)(NH_2)-COOH$$

from which either ammonia or water may be split off according to the direction of the reaction.

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[1] Knoop, F. Über den physiologischen Abbau der Säuren und die Synthese einiger Aminosäuren im Tierkorper. Zeitschrift für physiologische Chemie, Band 67, Heft 6, pp. 289–502. Strassburg, 1910. See p. 489.

It does not appear that Knoop actually experimented with ammonium salts, but Embden and Schmitz[1] in perfusion experiments on the liver have observed the formation of tyrosin, phenylalanin, and alanin, and probably of leucin, when the ammonium salts of corresponding acids were added to the perfused blood. The yield of alanin was especially large from pyruvic acid and less so from lactic acid, which corresponds with Knoop's view. When a liver rich in glycogen was perfused with blood containing ammonium chlorid, alanin was also obtained. This fact the authors explain as due to a formation of lactic acid from liver carbohydrate, and regard it as showing the possibility of the formation of an amino acid from ammonia and a carbohydrate. Even if the foregoing results are confirmed by further investigation, their significance for questions of nutrition may perhaps be questioned; but, nevertheless, the possibility of a synthesis of ammonia to amino acids, and thence to protein, by higher animals as well as by lower, should not be lost sight of.

RÉSUMÉ.

The results recorded in the foregoing pages may be briefly summarized as follows:

1. Amino acids and amids, which ordinarily constitute the larger part of the nonprotein of vegetable substances, are katabolized in the animal body, their nitrogen appearing in the urine.
2. In carnivora and omnivora neither the single substances of the foregoing groups nor the mixtures of them contained in plant extracts have been shown to be capable of performing the functions of protein.
3. In ruminants a conversion of nonprotein into protein appears to be effected by the micro-organisms of the digestive tract. The extent of this conversion appears to be relatively greater in the case of ammonium salts and asparagin than in that of vegetable extracts.
4. The protein formed thus from nonprotein seems to be digested subsequently. The apparent formation of indigestible protein observed by some investigators appears to be due to an increase in the metabolic products contained in the feces, caused by a specific action of the extracts upon the digestive tract.
5. By means of its conversion into bacterial protein, the nonprotein of feeds may serve indirectly for maintenance and also as a source of protein for milk, and probably for growth, in rations deficient in protein.
6. The limiting factor in the indirect utilization of the nonprotein of the feed appears to be the extent to which it can be converted into protein in the digestive tract rather than any inferior nutritive

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[1] Embden, Gustav, and Schmitz, Ernst. Über synthetische Bildung von Aminosäuren in der Leber. Biochemische Zeitschrift, Band 29, Heft 6, pp. 423–428. Berlin, 1910.

value of the protein thus formed as compared with that originally present in the feed.

7. The nonproteins are much inferior to the proteins in nutritive value for productive feeding. The prime effect of a substitution of nonproteins for proteins in the ration is a very marked falling off in the production. The indirect utilization of nonprotein simply serves to prevent this decrease from becoming as great as it otherwise would, and so in case of need to compensate partially for a deficiency of protein. On the other hand, with a reasonable supply of digestible protein the addition of nonprotein usually fails to increase the production of nitrogenous matter.

8. Recent experiments raise the question of the possibility of a direct utilization of ammonia as a source of protein by the higher animals.

CONCLUSIONS.

If the foregoing summary may be regarded as expressing with substantial accuracy the present state of our knowledge regarding the behavior of nonprotein in the animal body, what conclusions can be drawn from the facts there set forth as to the value to be assigned to this group in the computation of rations for farm animals?

VALUE FOR MAINTENANCE OF PROTEIN TISSUES.

It appears to be well established that nonprotein may be of equal value with protein for the maintenance of the protein tissues of the body, so far at least as this can be determined from the nitrogen balance.

Kellner's experiments on lambs (pp. 30–32) show qualitatively that ammonium salts and asparagin may perform the functions of protein in this respect, but they were present in excess and but a comparatively small proportion of them was utilized. In the experiments on cows reported by the Danish investigators (pp. 36–37) little or no protein was left after the demands of milk production were met, and the maintenance function must have been supported almost wholly by the nonprotein. Even assuming a minimum value for the maintenance requirement, there are a number of cases in which nearly or quite 100 per cent of the nonprotein appears to have been thus utilized, while in Kellner's experiment (p. 35) the available feed protein did not even equal that produced in the milk. In these cases at least, it seems necessary to conclude that a unit of nonprotein nitrogen in the ration was of equal value with a unit of protein nitrogen. Morgen's experimental results (pp. 32–35) occupy in this respect an intermediate position. They show that the nonprotein must have served for either maintenance or production, but a relatively small proportion of it (15 to 41 per cent) was utilized in this way.

VALUE FOR PRODUCTION.

With the exception of Kellner's and Morgen's experiments with ammonium acetate, there is as yet no positive evidence that nonprotein can replace protein for productive purposes, and ammonium salts do not occur in ordinary feeding stuffs in any considerable amount. In Morgen's experiments with plant extracts the supply of protein was more than sufficient in all cases to meet the demands of the diminished milk production. In the Danish experiments, with a relatively heavier milk production in the low protein periods, the limit was more nearly reached, but there can hardly be said to have been a significant deficit of protein in any instance.

As was stated on pages 32 and 35, it appears to the writer probable that the limiting factor in these cases was the extent to which the bacterial synthesis of protein was carried, rather than an inferiority in the nutritive value of the product. But however this may be, the practical result, from the standpoint of the computation of rations, is as if the nonprotein contained in rations such as are ordinarily fed may serve for maintenance but not for production. Whether a more extensive substitution of this type of nonprotein in place of protein would yield a different result can of course be decided only by experiment. It may be remarked, however, that the proportion of nonprotein to protein in the recorded experiments appears to be as great as it is likely to be in any ordinary ration, and, pending further evidence, it would seem to be the part of safety to consider that ordinarily not enough of the nonprotein is converted into protein (by bacterial action or otherwise) to make it of any material significance for the production of milk protein (and probably, therefore, of protein tissue).

THE COMPUTATION OF RATIONS.

If, however, the nonprotein is to be regarded as of full value for maintenance but as practically valueless for production, an undesirable complication is introduced into the computation of rations. The value of a feeding stuff (as regards protein) in a maintenance ration would be measured by its total nitrogen ("crude protein"), while the corresponding value of the same feeding stuff for productive purposes would be measured by its protein nitrogen.

But a considerable part of every productive ration serves for the maintenance of the animal. A part of the protein requirement, therefore, might be met indifferently by either protein or nonprotein, while for the remainder only protein would serve. For example, suppose a dairy cow to require per day 0.5 pound protein for maintenance and 1.75 pounds for the production of 35 pounds of average milk. She must be supplied with a ration containing a total of 2.25

pounds of digestible nitrogenous matter, in which, however, the nonprotein may vary from 0 to 0.5 pound, but may not exceed the latter limit. If, then, the "crude" protein (N×6.25) of the ration is made the basis of the computation and a ration is formulated supplying the necessary 2.15 pounds of digestible nitrogenous matter, a supplementary calculation would be required to determine whether or not the limit for nonprotein has been exceeded. Such a calculation, while it might signify little to the expert, would constitute an additional difficulty in the way of teaching the computation of rations to the practical farmer, who usually finds the subject sufficiently unfamiliar and complicated even when presented in the simplest possible way.

It is to be remarked in the first place that, so far as appears from the results cited in the foregoing pages, the nonprotein of feeding stuffs is available for the maintenance of ruminants only, while in the case of swine and probably of horses only the protein can be used for this purpose. In the case of the two latter species, therefore, it is evident that the digestible protein should be made the basis of the computation. Ultimately, of course, we should have separate tables of feed values for these animals, since their digestive capacity is in some respects materially different from that of ruminants. For the present, or as long as we continue to use a single table for all species of domestic animals, it seems undesirable to complicate the calculation in the case of ruminants by introducing the nonprotein into the calculation. Pending further investigation, therefore, it would appear to be the wisest course to continue to use ordinarily the digestible true protein as the basis of computing rations, ignoring the nonprotein. While doing this, however, it should be appreciated that rations thus computed will be likely to be unnecessarily high in protein, especially if composed largely of feeding stuffs rich in nonprotein, such as roots, silage, and green forage. This will be particularly the case when the protein requirement for maintenance constitutes a large proportion of the total protein requirement, as, for example, in working horses or in mature fattening animals; while, on the other hand, the error will be least with growing stock or good dairy cows where the productive quota is large as compared with the maintenance requirement.

If it is desired to make the computation more accurate in the case of ruminants, so as to avoid any excess of the relatively costly protein, it would appear that this end might be most simply reached by the method about to be suggested. This consists in formulating separately the protein requirement for maintenance and for productive purposes, computing a ration which shall supply sufficient true protein to meet the requirement for production, and then computing whether this ration contains sufficient nonprotein to cover the maintenance requirement.[1] Suppose, for example, it is desired to compute a ration for a 1,000-pound cow producing daily 35 pounds of average milk. Using for illustration the requirements formulated by the writer,[2] the ration of the animal should contain, for milk, 1.6 pounds protein and 9.6 therms energy value; for maintenance, 0.5 pound protein or nonprotein, and 6 therms energy value.

Disregarding in the first instance the nonprotein requirement, we may compute the following ration which supplies the necessary amounts of true protein and of energy:

Ration.	Dry matter.	Digestible protein.	Non-protein.	Energy.
	Pounds.	*Pounds.*	*Pound.*	*Therms.*
Silage, 40 pounds	10.2	0.26	0.21	6.64
Clover hay, 15 pounds	12.7	.80	.30	5.21
Corn chop, 5 pounds	4.2	.22	.02	3.60
Cottonseed meal, 1 pound	.9	.35	.02	.84
	28.0	1.63	.55	16.29

A supplementary calculation shows that the foregoing ration would also contain 0.55 pound nonprotein, the amount supplied by each feeding stuff being included for convenience in the foregoing table. The ration as computed thus proves to contain a slight excess of nonprotein over the estimated maintenance requirement, and may therefore be regarded as adequate, while if the total requirement for nitrogenous matter had been supplied by true protein it would have been necessary to use at least 1½ pounds more of cottonseed meal in the ration. While this method of computation adds slightly to the labor of computing rations for ruminants, it has the advantage of tending to economy in the use of protein concentrates, a thing which appears desirable since these are usually the expensive ingredients of the ration and since recent investigation indicates strongly that the protein requirements of animals have been more or less exaggerated in the current feeding standards.

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[1] For this purpose it would of course be necessary that the table used should show the percentage of nonprotein in the feeding stuffs in question.

[2] Armsby, Henry P. The computation of rations for farm animals by the use of energy values. U. S. Department of Agriculture, Farmers' Bulletin 346. Washington, 1909. See p. 19.

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36
U58

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258

FATTENING POULTRY.

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LETTER OF TRANSMITTAL.

United States Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., May 29, 1911.

Sir: I have the honor to transmit the accompanying manuscript entitled "Fattening Poultry," by Alfred R. Lee, of the Animal Husbandry Division of this bureau, and to recommend its publication in the bulletin series of the bureau. The work describes methods of feeding poultry on a large commercial scale, and presents figures on the cost of such feeding. Mr. Lee devoted the greater part of the past summer and fall to this work and collected complete data on the feeding of over 100,000 chickens. He also secured partial data on the feeding of upward of 200,000 others. The results obtained in dealing with these large numbers are unusually important because of the elimination of the errors which are peculiarly liable to occur in drawing conclusions from the feeding of a small or comparatively small number of fowls. The present investigation is, in fact, believed to be the first attempt to acquire comprehensive and reliable figures on the cost of producing a pound of gain in poultry.

The author desires to acknowledge the assistance rendered in the prosecution of the work by Messrs. Harry M. Lamon and C. L. Opperman, of the Animal Husbandry Division.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

Page

Introduction 7
    Educating the public taste 8
    Conditions in France and England 8
Methods of handling live poultry 9
Best breeds for fattening 11
    Comparison of breeds 11
    Individuality in chickens 12
The feeding season 14
    Length of feeding period 14
Methods of fattening 14
    Milk fattening 14
    Various methods in vogue 15
    Crate or trough fattening 16
Rations 17
    Grain mixtures 17
    Feather picking resulting from excessive grain feeding 18
    Milk or buttermilk essential in all rations 19
    The use of tallow 20
Mixing the feed 20
    Mixing machines 20
    Consistency of the feed 21
Number of times to feed daily 22
Color of milk-fed poultry 23
The feeding stations and their equipment 23
Details of the feeding experiments 31
    Experiment A 32
    Experiment B 37
        Comparison of experiments A and B 42
    Experiment C 42
    Experiment D 44
        Comparison of experiments C and D 46
Average daily consumption of grain per head 46
Daily death records 47
Fattening hens 48
Shrinkage in dressing 50
Cleaning and spraying the batteries 50
Poultry manure 51
Keeping records 52
Conclusions 53
Appendix 55
    Table I.—Details of feeding experiment A 55
    Table II.—Details of feeding experiment B 58

ILLUSTRATIONS.

PLATES.

Page.

PLATE I. Fig. 1.—Shipping crate for live poultry. Fig. 2.—Ordinary live-stock car, often used for shipping poultry. Fig. 3.—Live-poultry cars, generally used for long-distance hauls........................ 8
II. Fig. 1.—Portable feeding and mixing tank. Fig. 2.—Portable truck for moving birds. Fig. 3.—Manure truck........................ 28
III. Fig. 1.—Portable feeding battery—side view. Fig. 2.—Portable feeding battery—end view. Fig. 3.—Turkey-feeding battery. Fig. 4.—Two types of feed pails................................. 28
IV. Fig. 1.—Feeding station No. 2. Fig. 2.—Feeding station No. 3. Fig. 3.—Feeding station No. 4. Fig. 4.—Combination creamery and poultry-feeding station (station No. 5)........................ 28

TEXT FIGURES.

FIGURE 1.—Stationary feeding battery—end view........................ 24
2.—Stationary feeding battery—front view........................ 25

FATTENING POULTRY.

INTRODUCTION.

An opportunity for obtaining extensive and accurate data of the results secured in fattening poultry under commercial conditions in poultry-packing houses, which came up during the feeding season of 1910, was gladly made use of by the author, with the hope of throwing some light upon the cost of fattening poultry commercially, and also to show the relative gains which one can expect to secure with the different grades of poultry. Through the courtesy of two companies operating large poultry-packing houses in the Middle West it was possible to compare the results secured at different feeding stations and to study the efficiency of various methods, as well as the feeding value of different rations. The author is especially indebted to these poultry packers, who extended every possible courtesy to him and gave him free access to all the details of that part of their business in which he was interested.

The methods described are the result of years of experience and extensive, practical experimentation by these packers. As most of the experimental work hitherto published on poultry problems deals with small numbers, this careful study of a large number of birds suggested itself as a good opportunity to show what influence different factors may have in causing variation in results. The results of the season's work fulfilled the author's hopes to a considerable degree. The study of poultry problems under successful commercial conditions permits an investigation of the vital problems of the work without encountering the many difficulties which are liable to be met in starting a poultry plant for experimental purposes. This method also involves the use of large numbers of birds, thus eliminating many errors which may occur in drawing conclusions from small numbers.

Whatever value this work may have, aside from the comparative experimental data, lies in the fact that it describes in detail successful methods of fattening chickens for market and shows what was the cost of producing the gains. This work has been carried out successfully on a commercial scale in the Middle West, and as there is a tendency for the extension of poultry-packing houses southward, as well as a decided growth of the industry generally in the South. material on this subject should be of special value in that section. The normal growth of poultry interests in the Middle West develops a corresponding growth in the poultry packing and feeding business. Numerous experiments have been conducted showing the cost of producing a pound of gain in fattening steers, hogs, and sheep, but very little work has been published showing the cost of producing gain in poultry. The experimental work which has been published on fattening poultry has been of value rather as showing the comparative value of rations than the average cost of producing poultry flesh, as in many experiments the actual cost of producing the gains has been so high that it would not be commercially profitable.

In order to produce a superior quality of chicken flesh for high-class eastern and foreign buyers, managers of the poultry and egg packing houses of the Middle West for a number of years have fed "spring" chickens, producing the so-called "milk-fed" chickens. If these chickens were fed and fattened on the farm, it would not be necessary for the packer to put them into a feeding station, but the bulk of the chickens produced on the farm are too thin to make first-class dressed poultry without special fattening.

If the farmer gave his chickens a daily supply of grain, he could, in many cases, fatten his chickens at a profit before sending them to market, even when he sells to the large poultry buyer. He, of course, could not afford to put in an expensive equipment for fattening, but he could get his chickens in fair condition by supplying grain daily, or by confining those to be marketed for two or three weeks, and feeding freely either on corn, or on corn meal, wheat flour, or oat flour, mixed with skim milk or buttermilk.

EDUCATING THE PUBLIC TASTE.

When a bird has been properly fattened oil replaces much of the water in the flesh, so that when it is cooked the flesh becomes tender and juicy. Many consumers of poultry do not know how delicious a well-fattened spring chicken is, but after once securing a bird thus fattened they will most likely ask for the same quality in the future. As the people of this country become acquainted with the taste of chicken of good quality the demand will grow and they will be only too willing to pay for the extra cost of well-fattened birds. Most of the dressed spring chickens found in the average market to-day have been insufficiently fed; they can not be classed as fat.

CONDITIONS IN FRANCE AND ENGLAND.

The people of France and England have appreciated for some time the value of properly fattened poultry. Various methods of fattening are in use in both of these countries. In France most of the

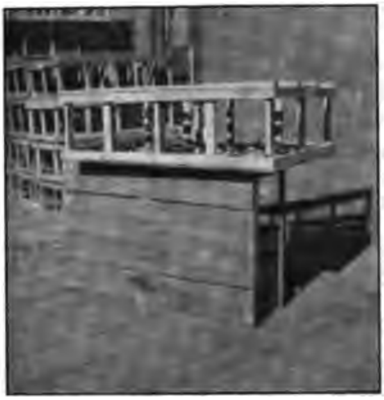

FIG. 1.—SHIPPING CRATE FOR LIVE POULTRY.

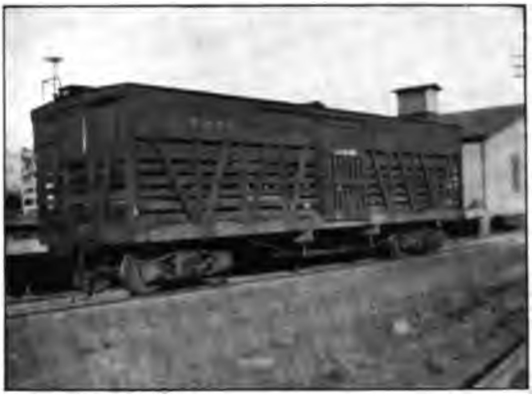

FIG. 2.—ORDINARY LIVE-STOCK CAR, OFTEN USED FOR SHIPPING POULTRY.

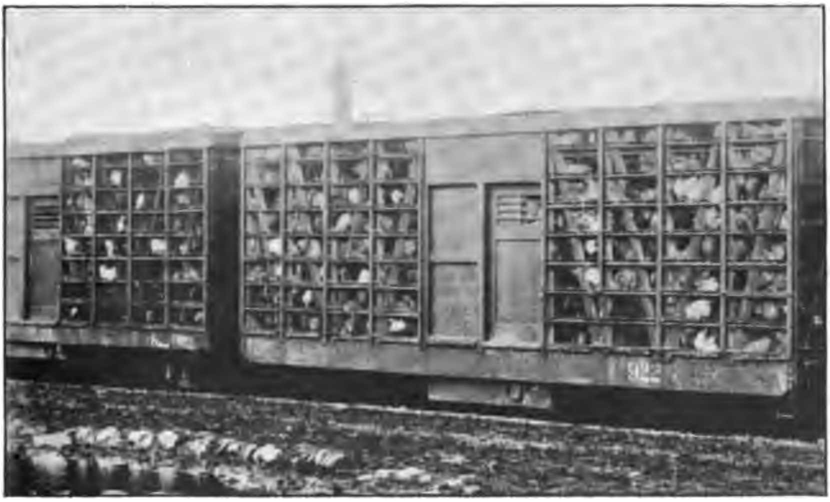

FIG. 3.—LIVE-POULTRY CARS, GENERALLY USED FOR LONG-DISTANCE HAULS.

poultry is fattened on the farms where it is raised by the farmer, who is usually very skillful in this art. Much of this fattening is done by hand, involving more labor than the American farmer can afford to give for this purpose, both on account of the higher cost of labor and of the lower price paid for the finished product in this country. Most of the special fattening in England is done at large establishments where the birds are confined in crates and fed from troughs for 7 to 10 days and then finished with cramming machines, making the total fattening period about 3 weeks. The coops or "batteries" in which the birds are fed are often placed out of doors, with some protection from the wind and rain. During cold weather the batteries are put into a building in order to conserve some of the body heat of the chickens; otherwise much of the value of the feed would be consumed in keeping the body warm rather than in producing flesh. Besides the extra care in feeding, special attention is given to selection and breeding, so as to build up strains which fatten readily. Much care is also taken in dressing the poultry, so that it is offered to the public in a very attractive condition. The farmers and special poultry keepers in this country could with good advantage adapt some of these methods to their own conditions.

METHODS OF HANDLING LIVE POULTRY.

Poultry buyers located in the towns and villages of the Middle West receive chickens from the farmers throughout the greater part of each year, but the stock used in commercial fattening is shipped in from June until the following December or January, the feeding season beginning earliest in the southern part of this section. The chickens are handled in many ways, often coming from the farm tied in lots of 4 to 6, with a tight cord around their legs or piled in burlap sacks, so that either their legs are scraped raw or become numb or they are half suffocated when they reach the local buyer or country merchant, who sells to the poultry buyer. It is difficult to understand the cruel and careless methods of some persons in handling their poultry, when by using a little care they could send their poultry to the buyer in good condition and save themselves money. The following styles of crates are used extensively by the packer and poultry buyer and could easily be adapted for use on the farm at a small cost and result in a great saving in labor as well as improving the condition of the poultry.

The coop shown in Plate I (fig. 1) is a very good type of wire crate, simple in construction and cheap. This crate is 2 feet 4 inches wide, 3 feet 10 inches long, and 13 inches deep, inside measurements, and weighs 28 pounds. The uprights are of furring ⅞ by 1¾ inches; the floor is solid and made of ½-inch boards and the sides and top covered with strips of 2-inch mesh wire, 1 foot wide, with a partition of similar material dividing the crate into two equal parts to prevent the birds from all bunching together. Shipping crates of about this size made entirely of wood weigh from 32 to 36 pounds, depending on the width of the wooden slats and the size of the corner posts, but they are no better than the wire crate and materially increase the weight, and consequently the cost of shipments. Wire crates of the type illustrated are also used for shipping turkeys, but are made 19 inches instead of 13 inches deep. Coops are also made of galvanized-iron strips and wire and of all kinds of modifications between those made entirely of wire and those made wholly of wood.

Some styles of crates have square wire doors on the top, while others have a thin slat which slides under three narrow strips of tin or iron and is fastened in the center with one nail. The coop illustrated is opened by moving a slat, fastened by a spring, which forces a small cut in the slat against a wire in the center. A coop thus shut can be opened easily and quickly, but occasionally gets out of order. There is a tendency to break the slats of coops which are nailed when opening them at the packing houses, especially if the work is done in a hurry.

The styles of coops above described are used in shipping either by express or by freight. The live poultry is often held for a day by the local poultry buyer and then shipped, either by express or freight, to the poultry and egg packer. If shipped by freight, an open car ordinarily used for shipping live stock locally is devoted entirely to eggs and poultry, which are picked up at each station and piled into the car, poultry generally at one end and eggs at the other. (See Plate I, fig. 2.) This car on arrival at the packing house is unloaded immediately if it comes in during regular working hours; if not, it is left till morning, although the eggs are often unloaded by the night force.

The birds are fed by the small poultry buyers if held for any length of time, and grain is scattered in the coops before they are shipped to prevent a heavy shrinkage in weight. The chickens are usually hungry by the time they are distributed in the feeding station and are not held long without feed. Chickens shipped in by express generally have less feed in their crops than those shipped by freight.

Poultry is also shipped extensively in cars built for live poultry (Plate I, fig. 3), especially when their destination is far enough away so that they will be over a day on the road. These cars, which are used extensively in interstate shipments, are of the following dimensions: 36 feet long, 9 feet 5 inches wide, inside measurements, with a "stateroom" 8 feet by 9 feet 6 inches in the center of each car and an aisle 2 feet 3 inches wide extending the entire length of the car. Each car has 8 tiers, which gives about 1,600 square feet of coop floor capacity, and each tier accommodates 16 coops. Allowing 36 fowls to each coop, cars of these dimensions will accommodate about 4,600 head, or about 18,000 pounds, of poultry, depending on the average size of the birds. About 2,000 to 2,400 geese or 1,200 to 1,500 turkeys make a carload. A water tank of 327 gallons capacity and a grain crib 8 feet square and 20 inches deep are attached to each car. All compartments have feed and water troughs accessible from the aisle, in which rations are fed consisting of corn meal, corn chop, and a small per cent of shorts in different proportions mixed with buttermilk. Dead birds can be easily seen and readily removed from all coops. These cars are well ventilated and carry the birds to their destination in good condition, the shrinkage rarely exceeding 5 per cent.

BEST BREEDS FOR FATTENING.

All varieties and types of chickens are fattened in this country, no special attention being devoted to developing strains or special types peculiarly adapted to produce a high quality of flesh or to give especially good results in fattening. Several breeds give good results in fattening, and these are preferred by men who make a specialty of feeding poultry in the following order: Plymouth Rocks, Wyandottes, Rhode Island Reds, or taken as a whole, birds of the general-purpose breeds. The feeders discourage the use of birds of the Mediterranean class, such as the Brown and White Leghorns and the Minorcas, because these birds average poorer results throughout the season in the feeding tests and they mature light, while the trade demands a heavy fowl. In order to make the farmer raise chickens of the first rather than of the second class mentioned, the packer, and consequently the smaller poultry buyer, often pays from 1 to 3 cents a pound less for light-weight hens.

More attention should be paid to buying poultry on a quality basis, thus showing the producer the gain which he may realize by keeping good poultry of the general-purpose breeds and giving the birds proper attention and feed before shipping to market. In some sections the packers have exchanged purebred cockerels of the general-purpose breeds for the mongrel and light-weight cocks kept by the farmers, thus rapidly improving the quality of stock in the localities where they obtain their supplies. The Orpingtons, various game crosses, and the Dorking make good poultry for fattening, but are not found in any appreciable numbers in the Middle West, although the Orpingtons have increased considerably in the last few years.

COMPARISON OF BREEDS.

Table 1 gives the gains secured in selected "batteries" in which the birds were sorted in feeding experiment A (see p. 32). It may here be stated that the coops in which the birds are kept during the feeding period are called batteries. There are two styles of batteries—stationary and portable—most feeding houses containing the stationary batteries. The per cent of gains shows that in general Leghorns make much poorer gains than Plymouth Rocks, but the results are not entirely consistent. The Leghorns make poor gains in the batteries after they are 2½ or 3 months old, as they are very restless, but they make good gains up to this age. Leghorns mixed with other birds in the compartments of the batteries tend to keep all of the birds excited. The chickens in Table 1 were all fed between September 22 and November 14.

TABLE 1.—*Results of fattening various breeds of chickens.*

Number of birds.	Breeding.			Days fed.	Average weight in.	Average weight out.	Died.	Average gain.	Gain.
	Rocks.	Leghorns.	Mixed.						
	Per cent.	*Per cent.*	*Per cent.*		*Pounds.*	*Pounds.*		*Pounds.*	*Per cent.*
68	100	....	....	14	3.68	4.25	....	0.57	15.5
192	100	....	....	14	4.03	4.82	....	.79	19.6
80	100	....	....	14	2.85	3.70	....	.85	29.8
80	100	....	....	9	4.01	4.61	....	.60	15.0
80	100	....	....	9	2.38	3.00	....	.62	26.1
80	100	....	....	7	3.26	3.78	....	.52	15.9
80	92	8	....	10	2.83	3.56	....	.73	25.8
80	87	....	13	8	3.18	3.85	....	.67	21.1
80	33	....	67	9	2.98	3.36	....	.38	12.8
80	....	100	....	9	2.88	3.19	1	.31	10.8
80	....	80	20	9	2.29	2.87	1	.58	25.3
80	....	65	35	6	2.16	2.55	....	.39	18.1
80	24	60	16	8	3.60	3.93	....	.33	9.2

INDIVIDUALITY IN CHICKENS.

Table 2 gives in detail the gains of the batteries making up two of the lots in feeding experiment A (p. 32). The first 11 batteries went on feed September 18 and the remaining 8 September 28. Records were kept of the gains of each battery. A lot includes all batteries put on feed on the same day and fed the same length of time. The batteries in the table are arranged in the relative order of the increasing average weights of the birds, but the percentage of gain does not vary directly with the average weight. The "Rocks" in this table were birds of the general-purpose classes, Barred Plymouth Rocks predominating. If the batteries were arranged in order according to the proportion of Leghorns the per cent of gains would still show no consistent relative order. These gains show a more consistent ratio between the average weight of the birds and the per cent of gain, which in general varies inversely. If the average weight is lowered by having a large proportion of Leghorns in the battery, this inverse ratio is not so apparent. This table shows the great variation in lots housed and fed alike, and emphasizes the great difference in the ability of the individual bird to put on flesh. This difference is greater because of the mixed stock in each battery. All the other lots in feeding table experiment A could be subdivided, and would show a similar variation in gains, but these two lots were selected as showing about the average variation within a lot. This emphasizes the error which is likely to occur in experimental work dealing with small lots, unless the chickens are of the same strain and have been handled alike from birth.

TABLE 2.—*Individuality in chickens.*

Number of birds.	Breeding.			Days fed.	Average weight in.	Average weight out.	Died.	Average gain.	Average gain.
	Leghorns.	Rocks.	Mixed.						
	Per cent.	*Per cent.*	*Per cent.*		*Pounds.*	*Pounds.*		*Pounds.*	*Per cent.*
80	10	90	....	8	1.89	2.24	....	0.35	18.5
80	55	45	....	8	1.98	2.41	1	.43	21.7
80	21	79	....	8	2.10	2.42	1	.32	15.2
80	35	65	....	8	2.13	2.33	....	.20	9.4
80	34	66	....	8	2.25	2.69	....	.44	19.6
80	16	84	....	8	2.31	2.83	....	.52	22.5
80	15	85	....	8	2.44	2.98	....	.54	22.1
80	9	91	....	8	2.46	3.05	1	.59	24.0
80	12	88	....	8	2.53	2.95	....	.42	16.6
80	12	88	....	8	2.70	3.09	....	.39	14.4
80	5	95	....	8	2.84	3.03	2	.19	6.7
80	....	65	35	6	1.60	2.20	....	.60	37.5
80	8	75	17	6	2.28	2.85	....	.57	25.0
80	29	13	58	6	2.35	2.83	....	.48	20.4
80	5	70	25	6	2.49	2.90	....	.41	16.5
80	10	58	32	6	2.50	2.81	1	.31	12.4
80	16	41	43	6	2.59	2.98	1	.39	15.1
80	....	58	42	6	2.59	3.00	1	.41	15.8
80	5	25	70	6	2.78	3.32	1	.54	19.4

The "feeder" who has charge of the fattening station often finds some in each lot of chickens that it would not pay to fatten. In such cases the Leghorns (especially the Single Comb Brown), Buff Cochins, Langshans, and all chickens with black legs are discarded. Varieties containing much Buff Cochin blood are claimed by some feeders to be very poor varieties to fatten. In sorting the birds the feeder sometimes throws out many birds in good market condition which it does not pay to feed under his conditions, as in many instances they would lose rather than gain in weight during the feeding period. If one can select the birds still more carefully for fattening he should pick out birds with short, stout, well-curved beaks, broad heads, bright, clear eyes, deep, broad breasts, and well-spread legs. Individual birds of the same breed vary greatly in their ability to put on flesh, but a strain can be selected and bred for this purpose. The superiority of certain strains of birds in England and France, in their ability to fatten readily, is quite marked. Crosses are frequently used in England for producing good fattening stock, but the majority of farmers and poultrymen in this country do not breed carefully and systematically enough to get good results from crossing, so that the offspring show lack of uniformity in type and size, which tends to lower the price paid for the birds. It is probable that feeders could with profit select birds along some of the lines mentioned in this paragraph, but this matter depends largely on each man's condition.

THE FEEDING SEASON.

The feeding season varies in different sections, depending on the climatic and seasonal conditions, and on the trade supplied with the dressed poultry. The season begins earlier in the South, generally in June, and lasts longer in the North, up to January or February, depending on the supply of live chickens. Many find it quite profitable to feed chickens as broilers during the early summer months, while other feeders prefer to feed the larger sizes, called "springs" and "roasters."

LENGTH OF FEEDING PERIOD.

The common practice in poultry packing houses is to feed each lot 17 days or less. The market or trade supplied and the results secured by the feeder determine the length of the feeding period. Most milk-fed chickens are fed for 14 days, but results secured in feeding indicate that a more profitable gain can be secured in a shorter feeding period, provided the same price per pound can be secured for the finished product. In England and Canada birds are fattened for at least 3 weeks, and if one uses a cramming machine it probably pays to feed for that length of time. If the birds are small or thin, they may be fed longer than heavier birds or those which are fairly well fleshed when they reach the feeding station. As the feeding season advances the tendency among feeders is to shorten the length of the feeding period, reducing it as low as 7 days in many cases. Many birds are merely "finished" by feeding for 5 to 6 days, and these are not generally classed as milk-fed poultry.

METHODS OF FATTENING.

MILK FATTENING.

Practically all of the special feeding in this country involves the use of milk, thus producing "milk-fed" chickens. These are also exported to some extent. Milk, while the least expensive, seems to be the most essential constituent of the ration, and when a feeder can not get milk in some form he generally does not attempt to fatten poultry commercially. The profit depends on various factors, many of which are local, and must be worked out by each individual. Among these factors are the supply and cost of the chickens, which depends largely on the competition of other buyers; the shipping facilities; the cost of the essential feeds; the availability and cost of efficient labor; the market, and the price which the packer can secure for his finished product. Often the packer has to feed his poultry to suit the demands of his market, but generally if a man has a high-class product he can make his own market, catering somewhat to popular fancies.

Besides these local factors there are certain essentials to success in a feeding station where poultry are fattened. First in importance is the manager of the station, or feeder, who must thoroughly understand all the details of the work and have a well-trained, observant eye, quick to note the condition and appetite of the stock. Success or failure depends primarily on this man, who must have the knack of caring for birds. The feeding station must be arranged to economize labor and to provide the best possible ventilation. Conditions must be of such a nature as to keep the birds quiet and contented, and at the same time cause them to consume a large amount of feed, in order to make profitable gains.

VARIOUS METHODS IN VOGUE.

Besides crate fattening from troughs there are several other methods in vogue, particularly in Europe. Among these are fattening by funnel, by machine, and by hand. The last is common in France, but can only be done economically where labor is cheap. The funnel method is used to some extent in England and France, with the funnel tube running directly to the crop, which is filled by pouring the mixture into the funnel. The other method, cramming by machine, is used extensively in England, generally to supplement trough feeding. The English feeder does not consider that the bird has been properly fattened until it has been finished with a cramming machine. Most of the large feeders have used cramming machines in the United States, but have not found them adapted to their conditions. There are two factors which may help to account for this attitude: First, very few feeders in this country have been able to use a cramming machine successfully and keep the birds contented; and, second, the trade has not been educated to the increased value of a machine-fed bird. However, the method is occasionally found in use where there is a special market for birds which have been crammed.

Some feeders in this country have obtained good results with the machine in one section, and made an absolute failure of the same method under different conditions. In England the art of fattening by machine is often handed down from father to son, thus producing first-class feeders. The cramming machine is used to some extent in this country for fattening hens which do not give good results on trough feeding.

In cramming, the birds are fed from 7 to 14 days from the troughs, and are then crammed twice daily for from 7 to 10 days, until they begin to go off feed, when they are marketed. The operator gauges the proper amount of feed to force into the birds by holding his hand on the bird's crop. If the crop is not almost or entirely empty at the next feeding time the bird is not given any additional feed.

Another method which is used to a considerable extent on a small scale in this country is pen fattening. This method is adapted for use on the farm, where the farmer does not care to go to the trouble of crate fattening, or where the price received for well-fed birds does not warrant the extra labor and feed cost of the latter method. Pen fattening has in some cases given very good results, but it is not as reliable as crate fattening, although the labor cost is less. It is used generally in fattening ducks. The quality of flesh secured by crate fattening is better than that obtained by pen fattening.

CRATE OR TROUGH FEEDING.

Crate fattening from troughs is the method of feeding employed in this country by most of the large fattening establishments. From 6 to 10 chickens are placed in the crate or battery, generally with a small amount of feed in their crops, and given a light feed at the next regular feeding period. Two methods are used in transferring the chickens from the coops to the feeding battery. The coops of chickens are weighed on scales located at some convenient place on the dock or in the packing-house building, and then put either into a portable transfer crate or directly into the portable feeding battery. If the birds are put directly into the portable battery, it saves the labor of rehandling and they go on feed in better condition than if rehandled. Many birds are graded into a lower class on account of broken wings, sometimes caused by handling after the birds have been on feed for some time; thus the use of the portable feeding battery tends to lessen the loss caused by rehandling. To facilitate the weighing of coops two strips of wood are nailed onto the scale platform, thus elevating the coops so that they do not touch the floor on any side, and making it easier to handle them. Some people advise dusting the birds before putting them into the batteries, but by keeping the batteries and coops clean and whitewashing frequently, the large feeders find that it is not necessary in the case of short feeding periods to dust the birds with lice powder.

From 6 to 10 birds are placed in each division of the battery, depending on the size of the birds and the ideas of the feeder. Ten birds seem rather a large number to place together, but very good results have been secured with this number in the portable feeding battery hereinafter described, although it would seem advisable to reduce the number to 8 when the birds weigh from 3½ to 4 pounds. Two or three chickens do better in a division together than when only 1 bird is placed in each compartment, and the cost of equipment and labor per bird varies inversely with the number of birds in each division.

RATIONS.

A perusal of the literature on the subject of chicken feeding indicates that there is a large variety of grain feeds, mixed in varying proportions, which are successfully used in fattening. But many of the large poultry feeders after trying various feeds and rations have found that a very simple ration, made up of only two or three grains, is best suited to economical gains under their conditions. Considering the large number of birds which they feed each year and the extent of their experimentation in feeding, it would appear that these simple rations must be of special merit for their conditions. Most rations are recommended for a feeding period of three weeks, although the length of the feeding period may influence the selection of the best ration. Birds fed only for a short time may be forced on highly concentrated feeds, whereas birds fed longer may need a ration containing a greater variety and less concentrated. While this may be true, many of the poultry packers feed the same ration to their chickens regardless of the length of the feeding period. The fact that it is easier to feed only one mixture may help to explain this condition, or it may be possible that the ration is not too heavy or concentrated even for the longer feeding periods.

GRAIN MIXTURES.

In selecting a ration the feeder must be influenced to some extent by the market price and supply of grains. Certain grains which are used for fattening are peculiarly adapted to local sections, and are not widely distributed at reasonable prices on the general markets. Among such grains are buckwheat, pea meal, graham flour, shredded-wheat waste, small potatoes, and in some places barley meal.

In the feeding records given in this bulletin the following rations were used: No. 1, 60 per cent corn meal and 40 per cent low-grade wheat flour; No. 2, 58 per cent corn meal, 36 per cent oat flour, and 6 per cent tallow, by weight. Ration No. 2 was varied during the season to suit the fancy of the feeder or the changes in the weather. This variation of the ration was not regular, but, generally speaking, as the season advanced and the weather became cooler a larger proportion of corn meal was fed, although the increase was not large if figured on the average per cent of corn meal in the ration each month. These two rations were selected by different individuals working under slightly different conditions. In general they are quite similar, except that tallow is present in one ration and not in the other. Many feeders after experimenting with a large number of different feeds have returned to these simple rations.

There are many other rations which have been used with good results, and perhaps are specially suited to certain localities on account of the relative price of grains. The following rations are mentioned as belonging to this class: No. 3, 2 parts of oat flour, 1 part of barley meal, and 1 part of corn meal; No. 4, 2 parts of oat flour, 1 part of barley meal, and 1 part of boiled potatoes; No. 5, 1 part of corn meal, 1 part of oat flour, and 1 part of wheat flour; No. 6, 2 parts of corn meal, 2 parts of buckwheat flour, and 1 part of ground oats; and No. 7, 1 part of oat meal, 1 part of graham flour, and 1 part of corn meal, by weight.

In France and England buckwheat flour, oat flour, and barley meal are used extensively in fattening. Shredded wheat waste has been used to replace oat or wheat flour, with good results, in places where it could be bought at a low price.

These rations include most, if not all, of the grains which are used extensively in this country, but there are many other combinations which have been, and are still, used for fattening.

All feeders are very particular that the grains used are of the best quality, and they find it especially necessary to watch the oat flour, often returning a shipment as unfit for their use. The oat flour, with the hulls removed, must be finely ground and should give a sweet taste when chewed. In some cases feeders have been forced to substitute other kinds of flour for the oat flour, as they could not always secure good quality oat flour, which is apt to contain other grains.

Grit is generally provided if the birds are kept on feed for two weeks or longer, giving 4 pounds of grit to 100 birds if fed twice a week. Where the birds are only fed a short time, 7 to 10 days, they do not need grit if they were raised in a section sufficiently supplied.

Clover or alfalfa meal, meat meal, blood meal, charcoal, and salt are often added to the rations, according to the fancy of the feeder. These constituents do not appear to be absolutely essential, but may be worth while for special conditions.

FEATHER PICKING RESULTING FROM EXCESSIVE GRAIN FEEDING.

Birds often become very restless on forced feeding of a highly concentrated ration, and commence feather eating and picking each other, often continuing until they have eaten a considerable part of the flesh of a live chicken. Probably an overheated condition of the blood, caused by consuming a large proportion of highly heating feed, such as corn meal, during hot weather will lead to habits of this kind. In such cases it may be advisable to reduce the proportion of corn meal and lighten the ration by adding some green feed, such as clover or alfalfa meal, and possibly a small amount of meat or blood meal. Salt, sulphur, or powdered borax, lightly sprinkled into the mash, have been suggested as remedies for this overheated condition of the blood. Salt is quite frequently used, but sulphur and borax, if fed in any appreciable quantity, appear to lessen slightly the appetite of the birds, although the difference is not marked. It is rather difficult to prove what effect these substances have in lessening the chance of the birds developing these bad habits; in any event these remedies are probably not used extensively.

MILK OR BUTTERMILK ESSENTIAL IN ALL RATIONS.

Milk is used entirely in mixing the various rations used in fattening. and is considered an essential ingredient, both in this country and in Europe. While good results may be secured without it, milk has such a beneficial effect on the birds that it is hard to get good results without using it. In some instances poultry shippers stopped feeding chickens when their supply of milk gave out. Buttermilk and skim milk are generally used, no particular notice being taken as to whether the milk is sweet or sour, but in almost all cases it was sour before it was fed to the birds. One large creamery in Kansas has experimented in condensing buttermilk, and they now manufacture a product which is put up in barrels, stored, and sold for feeding chickens. In one case they shipped carload lots to a feeding station located several hundred miles away. As many creameries have a large surplus of buttermilk during the spring months, this appears to be quite a profitable way of disposing of it, except that it involves expensive machinery, and that the condensed milk, being bulky, requires a large amount of storage space. This creamery was unable to satisfy half of the demand for condensed milk during the fall and early winter months. The milk is reduced to one-fourth of its original volume largely by evaporation, but part of the whey is drained off during this process. When condensed the milk is run into barrels without adding any preservative, and will keep indefinitely. Sample barrels have been kept for two or three years, and when opened the contents have been in good condition. Under ordinary trade conditions the milk would never be kept longer than one year. Fresh buttermilk, condensed buttermilk, and skim milk are preferred in this relative order. Whey is sometimes fed in addition to the condensed buttermilk, but it is too bulky and of too small feeding value to pay to move any considerable distance.

Various feeders have endeavored to find a substitute for milk, with little apparent success. Milk seems to have a very important influence on the digestive processes, keeping the bird in good condition under forced feeding. Beef broth has been used to some extent, with fair results, but it is not as good as milk. If the feed is mixed with water, from 5 to 15 per cent of the ration should be meat in some form, and vegetables or green feed should be added. Green feed is fed to some extent in very hot weather, but most feeders do not think that the results warrant its use. Finely ground beef scrap and meat meal are good forms of meat feed.

THE USE OF TALLOW.

Beef tallow is used by many feeders, but has been discarded by others, who claim that it produces a poorer quality of flesh. When only a very small amount is fed the difference in the flesh is not noticeable; but, considering the cost of the tallow and the possible poorer quality of flesh produced, it hardly seems to be an economical feed, although this depends largely on individual conditions, especially on the market to which the packer sells. In part of the feeding experiments in this bulletin about 6 per cent of the ration, excluding milk, consisted of tallow, and this had no apparent effect on the flesh. Tallow is often recommended to be fed during the last few days of the feeding period, but under ordinary commercial conditions it is hardly practicable to mix the feed separately and use it according to the number of days which the birds have been in the feeder. The tallow may be shaved directly into the feeding trough, but this method does not seem as practicable as to mix the melted tallow into the feed.

MIXING THE FEED.

The feed may be mixed with a rake or in a machine; some feeders preferring to mix with the rake regardless of the amount which has to be mixed. The feed can be mixed fairly quickly with a rake by a skillful feeder, but most feeders prefer to let a machine do the mixing where a large number of birds are fed. Some kind of power is necessary to run the mixer. When mixed by an iron rake the milk is run or poured into a large mixing tank and the grain added gradually, constantly stirring the mixture with the rake to prevent the formation of lumps, and to mix the different grains thoroughly. The feeder adds the different grains alternately, generally dumping in 100 pounds at a time, and mixing is continued until the mixture is of an even consistency. It is very necessary to have the feed free from lumps. Tallow may be kept in an open kettle heated by steam pipes, and gradually added to the feed in a melted state, after the milk and grain have been mixed. The pail in which the tallow is handled should be heated before it is used for the melted tallow, to prevent the liquid from congealing on the sides of the pail. The tallow is stirred thoroughly into the mixed feed.

MIXING MACHINES.

There are several styles of machines used for mixing the feed, each manager having his own ideas of the best kind of mixer. A horizontal mixer made of 2 tanks each 6 feet 6 inches long, 2 feet 6 inches wide at the top, and 2 feet 9 inches deep, containing a dasher running lengthwise of each tank, was used at station No. 4 with good results. There were 22 paddles on the horizontal shaft or dasher, set at different angles, each 13½ inches long, 1¼ inches wide, and three-eighths of an inch thick. When the machine was going these paddles barely missed the sides and bottom of the tank, which was concave on the bottom. The narrow side of the paddles cut the feed when in motion. The feed was held and mixed on a platform 15 feet by 6 feet, which was level with the top of the mixing tanks and was built flush against the tanks. The machine was made up of two sections, so that either one could be used separately. A single mixing machine built along similar lines, but upright instead of horizontal, was used at station No. 2, but this did not give very satisfactory results as the consistency of the feed varied when run out at the bottom of the tank. This tank had only one spout or valve, but later a second valve was inserted, which saved considerable time in filling the feeding pails. Each tank at station No. 4 had two valves for letting out the feed. A smaller mixing machine made in the form of a drum was used at two feeding stations not described in this bulletin. These drums opened on the side and could be dumped quickly, but their capacity was small. All of the tanks and mixing machines are fitted with steam pipes so that in cold weather the feed can be heated and fed while warm. If the feed is to be fed warm the milk is heated before adding the meal and flour.

CONSISTENCY OF THE FEED.

The feed is mixed to the consistency of thick cream, or so that it will drip from the tip of a wooden spoon. In very hot weather it is advisable to mix the feed thinner than in cooler weather, and results appear to indicate that one feed daily of a thin mixture with one or two thicker feeds makes the best feeding plan, although opinions differ on this point. The chickens seem to prefer the thicker feed, but it is apt to satisfy their appetites before they have consumed as much feed as they would if the mixture was thinner. This matter has to be left largely to the judgment of the feeder, but it should be observed carefully. As the birds receive no liquid except what they get in their feed, it is necessary to use quite a large proportion of milk in the feed. The percentage of milk used seems to depend on the kind of grains in the mixture, on the weather, and on the feeder. It varies from 55 to 70 per cent, and an average of 60 per cent or a trifle higher seems to give very good results.

The successful feeding of poultry depends largely on the ability of the feeder to notice the condition of the chickens on feed. Birds should be fed lightly for the first two or three feeds, gradually increasing the amount until they receive all they will eat up clean. The feed is poured into the troughs by the feeder, who walks rapidly through the aisles between the batteries, feeding a large number of birds in a short time. The condition of the birds when they go into the battery and the length of the feeding period have considerable influence on how soon to feed the birds the maximum amount. Ordinarily the birds are rather hungry when they go into the batteries, especially if they have been shipped in by express, and they can be fed quite freely from the first feed. If they have feed in their crops when put into the batteries, it is usual to feed very lightly for two or three feeds until they are quite hungry and have become accustomed to their new surroundings. Observations made on a considerable number of birds fed within a short time after they were put into the batteries showed that this practice was a good one under certain conditions, and that in many instances it was not advisable to feed a light ration as long as is ordinarily advised in fattening chickens. The main object in feeding should be to keep the birds' appetites keen and at the same time make them eat as much feed as they can assimilate.

NUMBER OF TIMES TO FEED DAILY.

Birds are fed from two to five times a day, but the more common practice is to feed either two or three times. A skillful feeder can get good results feeding twice daily and many prefer this method; but excellent results are secured by feeding three times a day, even by those who are not experienced feeders. An inexperienced person is apt to get better results by feeding three times a day rather than twice. Regular feeding is necessary, and if the birds are fed twice daily the intervals between the feeding times should be as nearly equal as possible. In this case it is well to feed at 6.30 a. m. and 3 p. m. If the birds are fed three times, feed at 6.30 a. m., 12 noon, and 4 p. m. The feeding hours must be regulated somewhat by the season of the year, by the appetite of the birds, and by the hours which the men are employed. By feeding a small amount often, the birds can be made to eat a larger quantity and their appetites kept keener. Each feeder must decide for himself whether there is enough to be gained by feeding oftener to pay for the extra labor involved.

A good many birds die when on feed, especially during certain seasons. The loss is greatest during hot summer weather, when the birds become prostrated with the heat; and later during October and November, when many of the birds develop some form of sickness. The batteries must be examined closely every day and sometimes twice daily, and all the dead or sick birds removed. While making the rounds for dead and sick birds some feeders find that other birds which are healthy but off feed may be removed and dressed at once, instead of keeping them on feed and having them lose in weight, or possibly become weak and sickly.

COLOR OF MILK-FED POULTRY.

A bleached appearance is very characteristic of milk-fed chickens. Milk is apparently the chief factor in causing this appearance, although the composition of the ration doubtless affects this point to some extent. The birds which were fed 14 days showed the effect of bleaching very plainly and a large proportion were white. Some, however, did not appear to bleach at all. The color in the lots fed from 6 to 9 days was more uneven, the birds showing streaks of yellow and white, although many were fairly white and even in color. Records kept of the comparative number of white and yellow birds at various intervals during the season in experiments A and B showed that in the former 73 per cent were white and 27 per cent yellow, while in the latter only 59 per cent were white and 41 per cent yellow. This would indicate that the use of low-grade wheat flour produced a whiter flesh than the oat flour, but there was considerably more milk in the ration of experiment A, and the larger proportion of milk may have influenced the color of the flesh more than the kind of grain. Allowing for this difference in milk it appears that the wheat flour tends to whiten the skin and flesh as much, if not more, than the oat flour. These records are not strictly comparable, as the dressed birds in experiment A, while uneven in color, were classed as white if they showed the effect of bleaching to any considerable extent. Butter color is sometimes added to the feed to give a rich yellow color to the flesh, but this was only done at one of the feeding stations in this bulletin, and only to a very few lots. Molasses was used for coloring during the season of 1909 at the same station and was said to have given a deep-yellow product. The packers stated that they did not care whether the dressed poultry was white or yellow except in a few lots where the market wanted a certain colored flesh.

THE FEEDING STATIONS AND THEIR EQUIPMENT.

Feeding stations, which are buildings used entirely or principally for fattening chickens, are operated in connection with most large poultry and egg packing houses, and are practicable in all large poultry producing sections where the farmer sells his poultry in relatively poor condition, provided the facilities for shipping or marketing are such that poultry can be held and shipped under cold storage conditions. These stations are generally located at or near a railroad junction or center in order that supplies may be drawn from a large territory. There are many different types of feeding stations, but all should be constructed to economize labor, to provide room for a large number of birds per square foot of floor space, and to keep the birds contented and healthy. The weakest point in most feeding stations is

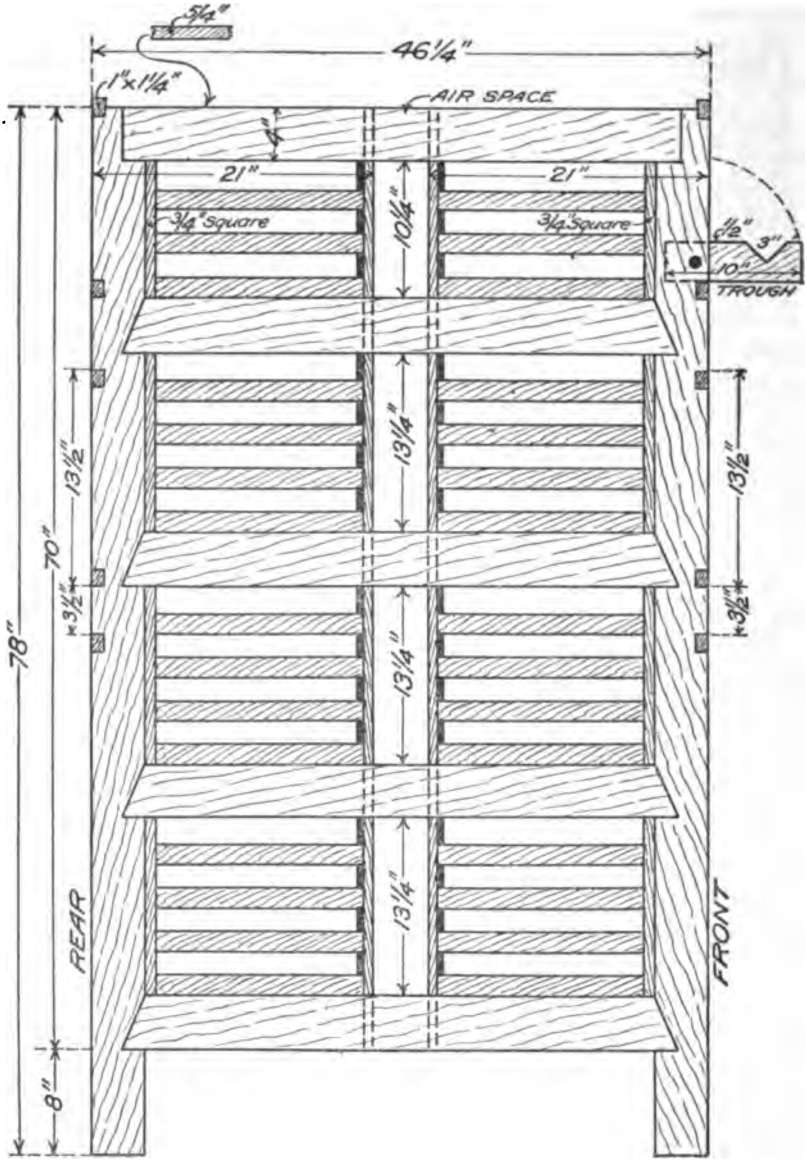

FIG. 1.—Stationary feeding battery, end view.

a lack of, or improper, ventilation. The following feeding stations were used for the experiments described in this bulletin; each one is designated by a number for convenience in reference;

STATION NO. 1.

This consisted of one section of a packing house known as the feeding station. The killing room where the poultry is dressed constitutes another section, and is situated on the side opposite the feeding station; the scales are located on the "dock" halfway between these two sections. This dock is a covered platform running the entire length of the packing house, and is devoted to the handling of the poultry and eggs. A spur track from the railroad runs parallel to the dock, so that the poultry and eggs can be unloaded directly from the car to the dock. The feeding station is 48 by 130 feet; 12 feet from the floor to the plate and 32 feet from the floor to the ridge, built on the "monitor" style. The walls are double, brick outside and plaster inside, and are sheltered by a projecting roof. The building contains 20 double-sash windows on both sides and 9 in both ends, with the sides of the monitor top entirely filled with sash and three double sash in both ends. A row of wooden shutters, 2½ by 2 feet, are placed on either side of the building just under the eaves. During hot weather the shutters are kept open and the windows all taken out and the openings covered with wire. The windows in the monitor top were originally hung at the top and opened in, but were changed about November 1 and hinged at the bottom. This change improved the ventilation in the house, as it tended to prevent the incoming air from falling directly on the birds in the batteries. About one half of one side of the house was built against another part of the packing house, and a dirt bank came up about 5 feet against one end, thus cutting off considerable ventilation during hot weather.

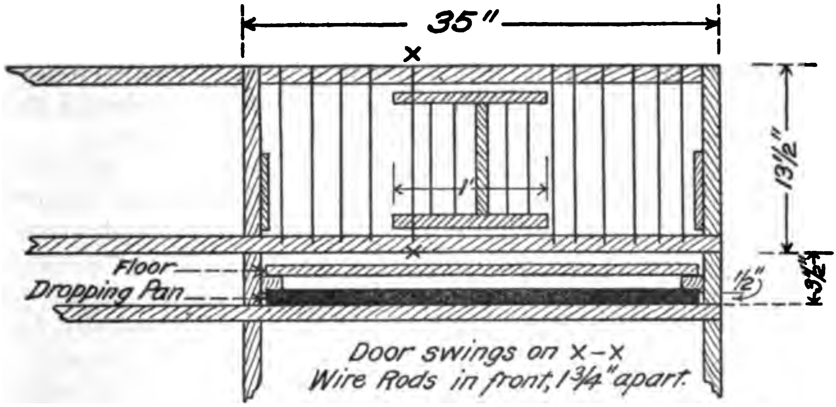

FIG. 2.—Front of stationary feeding battery.

This house was equipped with stationary batteries, four tiers high, as illustrated in text figures 1 and 2. Eight chickens or 6 hens were placed in each division, and as each battery contained 56 pens, it would hold 448 chickens, but generally 450 birds were put in each battery. Later in the season, when the chickens brought in would weigh from 3 to 4 pounds, only 6 birds were placed in each compartment. The floors of each tier were 1-inch mesh wire, such as is ordinarily used for confining chickens, with a roosting board 3 inches wide, 2 feet 10 inches long and 1 inch thick, laid across the center of each division and set in between nails, so that it could be easily removed. The sides of the battery are wooden slats or laths, with a space of 1¾ inches between them, with wire rods set in the center of the furring in the front of the battery. The dropping pan is made of galvanized iron with the edge turned up in front and slides under each compartment, 3½ inches below the wire floor. There is a 4-inch air space which divides the battery into two parts, as shown in the accompanying cut. Feeding troughs made of cypress, 2⅝ inches on the side and 3⅞ inches across the top, inside measurements, were hung along the front of each tier of coops, supported by wooden strips 10 by 3 inches, cut to fit the troughs, fastened with only one nail so that they can be pushed up into the battery when the troughs are removed. The center of the cut in this supporting strip is 2½ inches from the front of the battery, allowing about 1 inch between the front of the battery and the edge of the trough.

This house was arranged with a broad center aisle 8 feet wide, running lengthwise of the house, with side aisles between the batteries, which were 34 inches apart, and extended from the center aisle to the sides of the room. The side windows were at the end of the narrow aisles. This allowed the feeder to push the feed truck through the center aisle and one pail of feed would generally feed two tiers of birds, so that it only took four trips in each side aisle to feed the part of each battery which faces the aisle. This feeding station would accommodate 13,500 birds on feed at one time, with sufficient floor space for storing the grain and milk, mixing the feed, and storing the working equipment of the feeding station. The house was lighted by electric lights arranged so that the birds could see their feed on dark mornings and afternoons, and had four fans placed on top of the batteries to keep up a circulation of air in hot weather. These fans had to be cleaned frequently or they would become clogged with dust.

The feed was mixed in a portable feeding tank (illustrated in Pl. II, fig. 1), which is 2 feet wide, 6 feet 10 inches long on the top, 5 feet 6 inches long on the bottom, and 2 feet 1 inch deep. This tank is built of wood and contains a spout or valve at one end from which the feeding pails are filled. The truck ran on four wheels, a stationary pair at the end and two pivot wheels in front, so that it could be turned easily. The dropping pans were cleaned by scraping with a piece of galvanized iron 8 by 6 inches, one edge curved over to make a handle. A hook, made of bent wire, was used to pull out the droppings tray. The manure was scraped into a truck 21 inches wide, 4 feet long on the bottom, 4 feet 9 inches on the top, and 16 inches deep, with a removable slat across the top to rest the tray on while it was being cleaned. This truck contained one small pivot wheel on either end, with a pair of larger stationary wheels in the center. The portable crate or truck used for moving the birds is shown in figure 2 of Plate II. This is divided into two parts, with feed troughs in the center, and is covered with 2-inch mesh poultry wire. The crate is 5 feet 5 inches long, 5 feet 2 inches high, and 3 feet 2 inches wide, with doors hinged at the top, which swing in and are held open by a curved hook shaped like a letter U.

STATION NO. 2.

Feeding station No. 2, shown in Plate IV, is entirely separate from the other sections of the packing house. The building is 42 feet wide by 120 feet long, 14 feet to the eaves and about 15 feet to the top of the building proper, which has a monitor top 9 feet wide extending 5 feet 6 inches above it. Each side of the monitor top is divided into two tiers of shutters, 26 in each tier, hinged at the top, which swing out. Each side has 18 single-sash windows, set about 2 feet below the eaves, and 6 sash in either end. There are two tiers of shutters 2 feet 9 inches wide extending around three sides of the house, just above the floor, which are hung at the top and swing out. The east side—the building running north and south—has doors which slide up toward the eaves in place of the shutters. These shutters when tilted out shade the building during hot, sunny days. All of the shutters are made of ½-inch matched lumber, and the sides of the house are built of ship lap ¾ inch thick and 5½ inches wide.

Many of the birds in this house were sick, owing in some cases to their condition when they reached the packing house, but in most cases to drafts in the feeding station. The shutters had warped somewhat and did not fit tightly, so that the house was very drafty, as it was customary to keep part of the shutters open in the monitor top. This is a good house for summer feeding, as it can be thrown almost entirely open, so as to get all of the fresh air possible, but it is built too cheaply to make a good house for feeding in cold weather. This is quite apparent from the record of the deaths and of the gains obtained. If the shutters in the monitor top were hung at the bottom and swung in, the air would not fall directly on the birds below. During November heavy duck cloth was stretched partly across the building, about 2 feet above the top of the batteries, to cut off the drafts, which improved the house but did not allow sufficient ventilation. This style of house should be built so that the shutters on the side could be closed absolutely tight in cold weather and the draft cut off from the monitor top. Some ventilation is necessary even in cold weather to keep the air fresh.

This station was equipped originally with stationary batteries, but most of these had been replaced with portable feeding batteries, as shown in Plate III. This battery is divided into 8 coops, 4 tiers of 2 coops each, and holds 80 springers or 64 hens. It is 2 feet 7½ inches wide and 5 feet 9 inches high. The slats in the front are 1⅜ inches apart and each set of slats, which is 8¼ inches wide, is held in by buttons, so that it can be easily removed and a set of slats which are closer together or farther apart may be quickly inserted. As the sizes of the chicken's heads vary considerably during the season this changeable front is of value. The dropping pans are 1¾ inches below the floors, which are made of heavy, square-mesh wire, and have roost boards 2 inches wide by ¾ inch thick by 2 feet 6 inches long. The bottom of the first floor is 6 inches from the ground, and it is 15 inches from the wire floor to the top of each coop, making each tier, including the dropping pans, 16¾ inches deep. The battery rolls on four wheels, two double-pivot wheels in front and two wheels connected by a bar in the rear. The sliding doors on the sides are fitted with hooks which fasten into eyes on the battery. The whole battery is made of furring, 1⅜ by ⅞ inches, covered with 2-inch mesh wire and laths. The feeding troughs are 3½ inches across the top, inside measurement, and 3 inches from the top edge to the bottom, outside measurement. These troughs are held in place with bent wires which are flexible so that they give if the troughs hit any obstacle, thus preventing breakage. A wire partition divides the battery into two equal parts. A similar battery is used for feeding turkeys except that it contains three tiers instead of four and the slats in front are 2⅝ inches apart. This battery is illustrated in Plate III, figure 3.

These coops were arranged in long rows running lengthwise of the house, spaced about 4 inches apart in the rows, with the rows 3½ feet wide. This arrangement was changed to suit the varying conditions. The arrangement of the batteries in long rows tended to waste labor, as the attendant would feed down a row till he emptied his pail and then return to the end for another pail of feed, thus making many trips with an empty pail. This could be overcome by arranging the batteries differently, or by having feed at both ends of the rows. The batteries of birds to go on feed were pushed in at one door and rolled out at the other end, which made it necessary to keep moving the batteries in each line down toward the end of the house. This frequent moving is detrimental to good results in feeding as it keeps the birds restless.

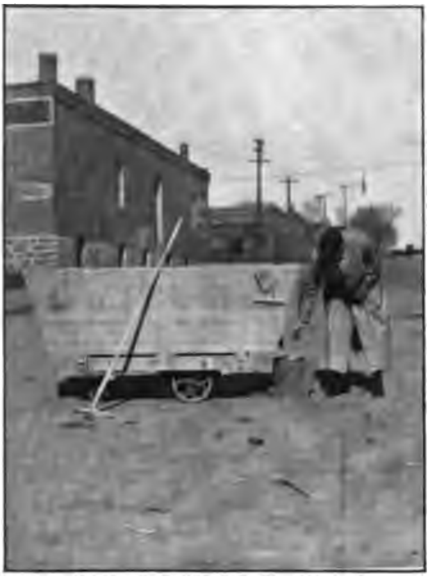

FIG. 1.—PORTABLE FEEDING AND MIXING TANK. NOTE FEEDING PAIL.

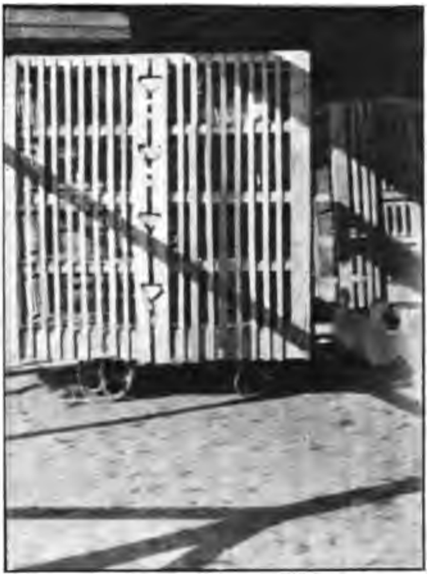

FIG. 2.—PORTABLE TRUCK FOR MOVING BIRDS.

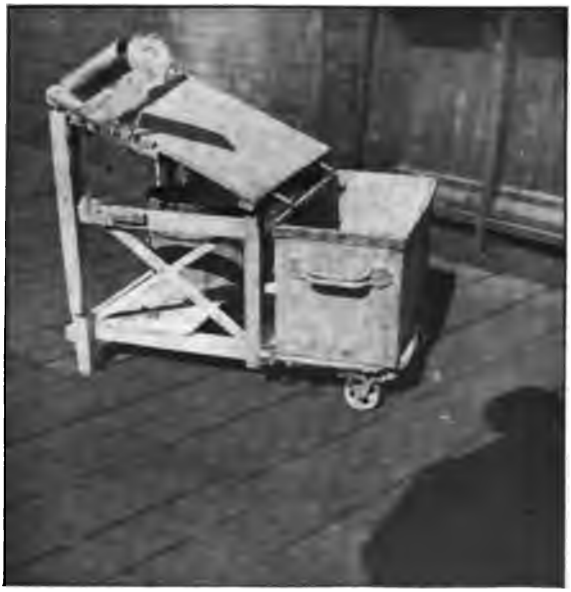

FIG. 3.—MANURE TRUCK.

M10U

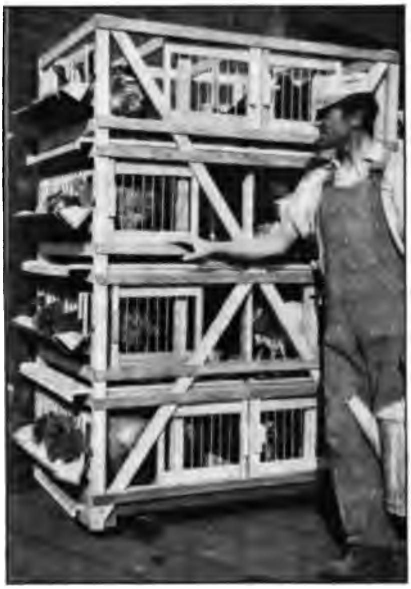

FIG. 1.—PORTABLE FEEDING BATTERY; SIDE VIEW.

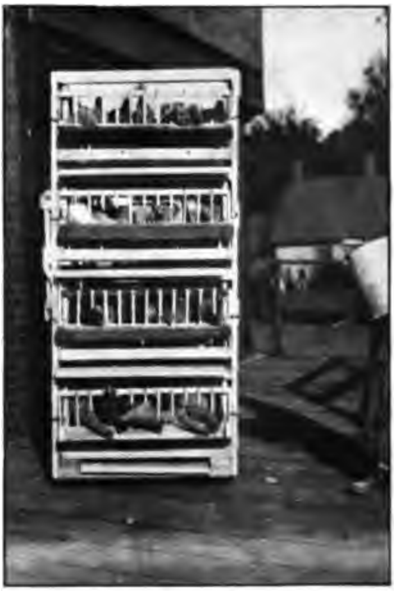

FIG. 2.—PORTABLE FEEDING BATTERY; END VIEW.

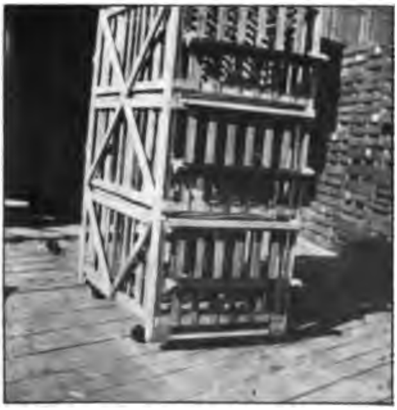

FIG. 3.—TURKEY-FEEDING BATTERY.

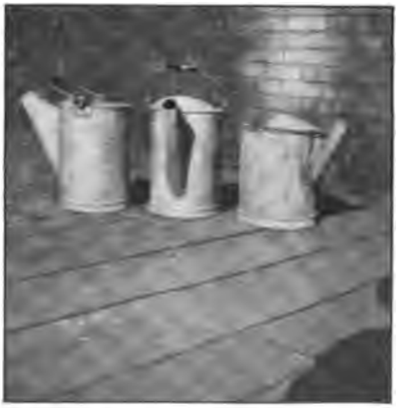

FIG. 4.—TWO TYPES OF FEED PAILS.

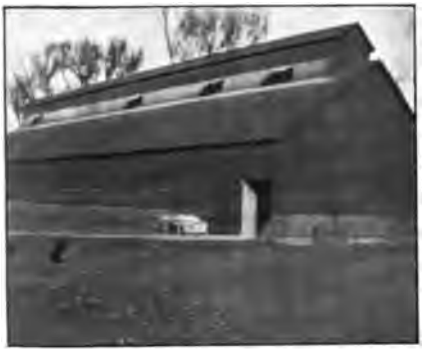

FIG. 1.—FEEDING STATION NO. 2.

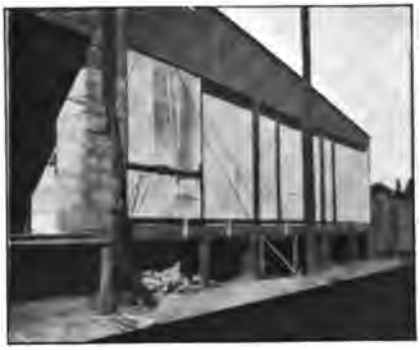

FIG. 2.—FEEDING STATION NO. 3.

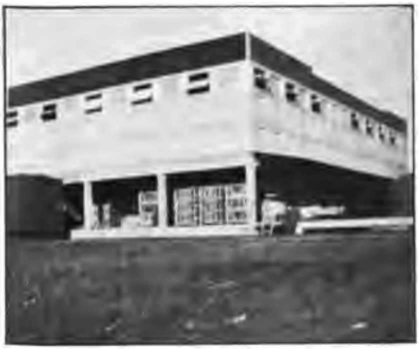

FIG. 3.—FEEDING STATION NO. 4.

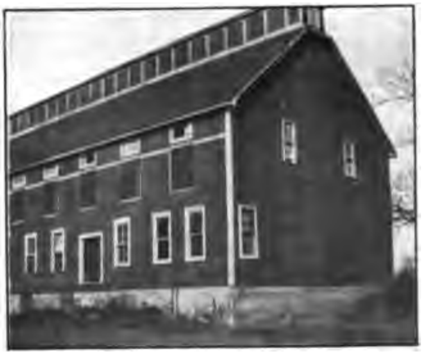

FIG. 4.—COMBINATION CREAMERY AND POULTRY-FEEDING STATION (STATION NO. 5).

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Various styles of pails were used in feeding, two good types of which are shown in Plate III, figure 4. These pails hold about 14 quarts.

The best style for a feeding pail depends somewhat on the thickness of the feed and the style of the battery in use; the styles in Plate III, figure 4, were adapted to the portable feeding batteries and were used at stations 2, 3, and 4. The feeding pail used at station 1 had a blunt snout 4 inches wide, which projected out 4 inches at the top and tapered to nothing at the bottom of the pail. This held 18 quarts and had to be handled carefully or the feed would come out too quickly. It would not be adapted to use with portable batteries, as much feed would be wasted between the troughs. The spout of the shorter bucket, illustrated in Plate III, figure 4, is too small for thick feed and makes slow feeding, but the other style of bucket is very satisfactory. This bucket is 12½ inches deep and 9 inches in diameter. The small end of the spout is 1⅝ inches in diameter. Some pails have a handle on the back near the bottom of the pail.

The manure truck shown in figure 3 of Plate II was used at stations 2, 3, and 4. All of the stations were equipped with some kind of spraying machine, fitted with small air-pressure tanks, which were operated by hand power. A small amount of carbolic acid or some similar disinfectant was generally mixed with the whitewash.

STATION NO. 3.

The best results were secured in feeding station No. 3, which is shown in Plate IV, figure 2. This is a lean-to, built against one side of the packing house, and is a very inexpensive building. The floor is a single layer of unmatched boards, and the sides are of standard duck cloth, 10-ounce weight. The curtains overlapped each other about a foot and were arranged so that they could be easily rolled up and down. Three sides of the house could thus be entirely opened to allow perfect ventilation. The floor should either be double or else made of matched lumber, as the single unmatched boarding allows too many cracks. This shed was protected on all sides by other buildings, so that it did not get the full force of the wind. The birds were very free from colds and disease in this building, and it appeared to be an ideal place for summer and early fall feeding.

The curtain idea could be adapted to other feeding stations with good results, if care is taken to see that the house is made free from drafts. This house did not contain any windows. The floor was 45 by 93 feet, with the roof 9 feet from the floor on the lower side and 13 feet from the floor where it was attached to the packing house. The house was equipped with portable feeding batteries arranged in short rows with a center aisle 5 feet wide running the long way of the building and side aisles 4 feet 6 inches in width. These batteries were changed to suit conditions. This station was separate from the other parts of the packing house, so that the birds were disturbed only at feeding and cleaning times. The feed was mixed in a long stationary mixing tank and carried to the feeding room in pails on trucks.

STATION NO. 4.

The entire second floor of the packing house was built for a feeding room in station No. 4. This is a new station and contains some very good ideas. It is illustrated in Plate IV, figure 3. This feeding room is built with a wing; the main part of the house is 48 by 140 feet with the wing 48 by 48 feet. The house is equipped with portable batteries similar to those at stations 2 and 3, and arranged as at station 3, with a center aisle 4 feet wide and side aisles 3 feet 10 inches wide, but subject to change according to conditions. This station was equipped with a mixing machine (described on page 20) and had an elevator large enough to hold two portable feeding batteries, which connected with the killing and weighing room on the first floor. The first floor was divided into a killing or picking room, a packing and small cold-storage room, an office, and general space for weighing, storing equipment, etc. Both floors of this building were made of cement.

The feeding room has an almost flat roof about 15 feet from the floor, and all sides of the room except the west contain two tiers of shutters each 4 feet 3 inches high and one tier of windows 3 feet high, so that the room can be thrown almost entirely open. The west side contains one tier of shutters and one of windows. The shutters are hung at the top and swing out from the bottom, while the windows, which are glazed, are hung in the center. The shutters are made of narrow strips ⅞ inch thick, laid diagonally, and the whole room is well built. This station was not occupied until November, but it is apparently a very good type of feeding station and contains many excellent features. The ventilation in warm weather ought to be ideal and yet the building can be shut absolutely tight in cold weather if desired.

The birds were moved into this station from station No. 5, shown in figure 4 of Plate IV.

STATION NO. 5.

Station No. 5 is a combination of feeding station and creamery, the second floor being the feeding station. This house was equipped with long stationary batteries, built somewhat similar to those described in station No. 1, except that the batteries were only three tiers high. The wires in the front of the coops were 1¾ inches apart. The center aisles were 3 feet 9 inches and the side aisles 2 feet 6 inches wide.

DETAILS OF THE FEEDING EXPERIMENTS.

The records which are shown in detail in Tables I and II of the Appendix represent results obtained at different feeding stations in the Middle West during the season of 1910. The tests cover a large number of birds, and the conclusion derived from the averages should be of considerable value, and should largely eliminate the error which is almost certain to be made in drawing conclusions from experiments dealing with small lots. The variation found to occur within a lot kept under similar conditions during the feeding test clearly indicates how great the error may be if conclusions are drawn from results secured in dealing with small numbers. Undoubtedly, however, the conditions existing at each feeding station, outside of the rations used, had considerable influence on the gains secured.

Experiments A and B give the results of each lot in detail, while experiments C and D only give the number of birds, the length of the feeding periods, and the gains in terms of percentages. The total weight of the birds before they were fed in these records is 793,359 pounds, 303,222 pounds of which are included in the detailed experiments A and B.

In experiment A the number of dead is the difference between the "Number in" and the "Number out," but in experiment B a large number of crippled birds were removed and dressed, as is more fully explained hereafter, and their weight is credited to the particular lot in the column headed "Gain" and in "Per cent of gain," but is not included in the "Weight out" column. The column headed "Average grain daily per head" represents the average daily feed (not including milk) of each lot for its feeding period. This factor is obtained from the daily feeding reports which give the total daily consumption and the number of all the birds on feed for each day, and is based on the assumption that all the birds eat the same amount of feed. The total feed for each lot is derived from this factor. This method of obtaining the total feed is not absolutely correct for each lot, but it is the only method which is practicable under commercial conditions which deal with large numbers, and the possible error would not affect the averages, but would help to explain the differences in the gain of certain lots which probably averaged to eat different amounts of feed per bird.

The following prices were used in figuring the feed cost of grain:

Corn meal	$1.35	per 100 pounds.
Low-grade wheat flour	1.35	per 100 pounds.
Oat flour	2.20	per 100 pounds.
Tallow	.08	per pound.
Buttermilk	.015	per gallon.
Condensed buttermilk	.75	per 100 pounds.

The average daily labor cost in experiment A was $7.29 per 10,000 birds on feed, which constant was used throughout the feeding season. The labor cost per 10,000 head varied considerably during the season in experiment B and the following constants were used: $9.44 per 10,000 head for lots 1 through 19; $9.53 for lots 20 through 41; $12.06 for lots 42 through 63, and $15.63 for lots 64 through 83. The cost of 100 pounds of grain, plus the cost of the milk used in feeding that amount of grain, varied as follows in experiment A: $1.93 per 100 pounds of grain for lots 1 to 20; $2.06 per 100 pounds for lots 21 to 37, and $1.95 per 100 pounds for lots 38 to 63. The cost in experiment B was $2.31 per 100 pounds of grain for lots 1 through 19; $2.30 for lots 20 through 41; $2.43 for lots 42 through 63; and $2.44 for lots 64 through 83. The grain in experiment A cost $1.35 per 100 pounds throughout the feeding period, while the cost of the milk used with 100 pounds of grain varied from $0.585 to $0.709. The cost of 100 pounds of grain in experiment B varied during the feeding season from $1.94 to $2.17, and the cost of the milk used with 100 pounds of grain varied from $0.275 to $0.352. Condensed buttermilk was fed in experiment A in a much thicker state than ordinary buttermilk, which explains the increased cost of the milk in experiment A over that in experiment B. The average cost of 100 pounds of grain in experiment A for the entire season was $1.35; and in experiment B, $2.06; while the average cost of the grain and the milk per 100 pounds of grain was $1.98 in experiment A and $2.37 in experiment B.

EXPERIMENT A.

The feeding was conducted at station No. 3. All of the lots in this experiment were fed alike except that the length of the period varied from 6 to 10 days. These birds were only fed for this short period because the existing conditions were such that it was not convenient to keep them on feed for a longer time. The gains secured indicate that under good conditions a large gain can be made in a short time.

Stock.—The lots were composed of stock of mixed origin put into the feeding batteries just as they came in from the small live-poultry buyers, without sorting. Each lot contained birds of various weights, but, as the records show, the average size or weight increased as the feeding season advanced, although there were some birds of broiler size in practically all of the lots. The Barred Rocks were the most popular breed; records kept at various intervals showed that about 42 per cent were Plymouth Rocks and 17 per cent were Leghorns. None of the other breeds were represented by large numbers of birds, but numerically they were present in the following order: Wyandottes, Rhode Island Reds, Orpingtons, Minorcas, and Langshans, constituting altogether not over 10 per cent of the total number of birds on feed. Thus 31 per cent of the birds were of mixed breeding. These figures only represent percentages in the rough, as the birds were classed as Plymouth Rocks, Leghorns, etc., if they had the most prominent characteristics of these breeds; many of them were probably grade stock.

These figures, moreover, represent averages of large numbers, and do not necessarily represent the actual composition of any specific lot.

The average quality of the stock in experiment A was good, apparently slightly better than in experiment B and considerably above that in experiments C and D. This means that the majority of the birds were slightly better fleshed and that there were fewer sick birds than in the other experiments. The difference in the health of the stock was hardly apparent when the birds were received at the feeding stations, but showed up noticeably during the feeding period, although the housing and management undoubtedly affected the health at the different stations, thus introducing a new factor, which must be considered in making comparisons.

Handling.—Most of the birds in experiment A were shipped by express to the feeding station, not over one-third arriving by freight. In general the distance shipped was short. The birds shipped in by express were weighed and put into the portable feeding batteries, previously described, shortly after reaching the feeding station. Those shipped by freight were handled in crates in stock cars, and generally stood several hours at the packing house before they were unloaded and put into the feeding batteries. The use of a portable feeding battery eliminates labor to a considerable extent, and involves less handling of the birds, both when they go into the feeder and when they come out. The birds undoubtedly get into the feeder in better condition, and there is less chance of breaking the wings after they leave the battery. After fattening, the batteries of birds were taken directly into the killing room, where they were taken out by the pickers as killed.

Feed.—Ration No. 1 was fed to all of these birds, the grain and buttermilk being mixed with a rake in a large tank. The buttermilk used was condensed and was diluted with about 2 parts of water to 1 of buttermilk. A small amount of whey and considerable skim milk was fed during the season, replacing some of the water, and, at times, a part of the buttermilk when the supply ran short, as happened during parts of October and November. Granulated or shredded curd was added to the ration several times during September and October, and the birds appeared to relish it very much. No grit was provided for the birds in this experiment.

The combination of condensed buttermilk and skim milk with the grain made a very thick feed, which was eaten very eagerly by the chickens, but it was necessary to give a thinner feed once each day, which was done by increasing the proportion of water. The chickens were not so eager for the feed when whey was used to replace much of the buttermilk, which often happened during October and November.

Method of feeding.—The chickens were fed three times daily, generally receiving the thinner feed at noon. Wheat flour has to be added very gradually in making the mixture, otherwise it will lump, and the person who mixed the feed often found it necessary to knead it as the housewife kneads dough in making bread. The feed was run into pails, which were carried into and through the feeding room on trucks. Generally two or three persons handled the feed, one filling and distributing the pails while the others did the feeding. One man fed part of the birds regularly three times a day, but he had different assistants at various times during the feeding season, no other help being kept specially for this work. The results, as shown in the record, indicate how well this system of feeding is adapted to conditions where the help is not especially experienced in feeding chickens. A moderate amount of feed was poured into each trough, and by the time the feeders had fed all the birds once those fed first had eaten up their supply and were looking for more. The feeder then gave a second feed, which was a light one, only to those birds that had cleaned up their first feed. Generally 20 to 30 minutes intervened between the first and second feed, according to the length of time it took the feeders to go through all of the aisles.

Sometimes the feeder would go around the third time, but not generally. This method of feeding appears to stimulate the appetites of the birds so that they consume a large amount of feed; and it does not require as skilled a feeder as is necessary when the birds are fed only once. There is also less chance of feed being left over in the troughs. The feeder went through the batteries about one hour after feeding, removing any feed which was not cleaned up by that time. However, if care is exercised with this method of feeding there should be no feed left over to be cleaned up. When the birds clean the feed up quickly the troughs are left in good condition, but if any feed is left over the feeder is apt to leave a little in the trough when he scrapes out the surplus, and this is likely to ferment or become sour. Therefore, the aim should be to feed only what the chickens will eat up clean. When the feeding is managed properly the feeding troughs appear as though they had been washed after the birds are through eating. The feeding troughs are not washed under ordinary commercial conditions except in special cases. When the batteries are sprayed the troughs receive more or less whitewash, but no special care is taken to clean them, as they are not dirty ordinarily.

The batteries were arranged to suit the convenience of the operator and to conform to the size of the room. The aim was to give the birds the best possible conditions without making the labor cost excessive. During the first part of the feeding season most of the birds were fed in a general purpose room, used for weighing all of the live and dressed poultry, for storing and mixing the feed, for batteries of hens held only one or two days before killing, and for candling eggs. In other words, there was something going on in this room practically all of the time, still the birds made good gains. During this time a few of the batteries were kept on the open dock where there happened to be a little available space. A new feeding shed was built, into which the batteries were moved on October 22, where the birds were isolated and so were disturbed only at the feeding times. There was no marked change in the gains due to this change in the feeding rooms, which appears to indicate that while it may be better to have the birds in a quiet, secluded room, this point is not as essential as is generally supposed. Most articles on fattening recommend that the feeding station be kept dark except at feeding times, but many of the large poultry packers pay no attention to this matter, and results appear to indicate that it has no important bearing on the fattening question. It is impossible to draw definite conclusions about such questions from the differences in the gains, as there are so many other factors subject to constant variation which affect the results.

The batteries were arranged in lines with an aisle running through the center of the building from end to end, as described under station No. 3 (p. 30). Batteries put on feed at the same time were kept together for convenience in handling. The batteries were placed several inches apart to allow a good circulation of air, the distance apart depending both on the temperature and on the amount of available floor space. One great advantage of the portable batteries is that they may be spread around to suit varying conditions. As birds often suffer from the heat during excessively hot weather, and many deaths occur, this point affects gains quite materially. The influence of the plan of the house on the gains is discussed more in detail under "Feeding stations." These birds were housed in station No. 3 after October 27. The batteries should also be arranged for convenience in feeding, so that a pail will contain feed enough to go up and down each side aisle and bring the feeder back to his source of supply, thus avoiding a waste of time in carrying an empty pail. The method employed in this house was to have a wide center aisle with branch aisles five or six batteries deep.

Table I of the Appendix shows the results of the feeding in experiment A in detail; a summary is given in Table 3, below.

Table 3.—*Summary of feeding experiment A, arranged according to length of feeding period.*

Number of head.	Days fed.	Average weight.	Per cent of gain.			Grain per pound of gain.		
			High.	Low.	Average.	High.	Low.	Average.
		Pounds.	*Per ct.*	*Per ct.*	*Per ct.*	*Pounds.*	*Pounds.*	*Pounds.*
2,068	10	2.51	23.5	11.5	18.5	5.32	3.01	4.04
10,360	9	2.40	26.1	11.2	19.4	5.10	2.55	3.52
11,878	8	2.55	27.1	10.9	17.2	4.40	2.17	3.37
15,731	7	2.39	29.6	11.4	19.2	4.55	1.92	2.68
3,907	6	2.18	18.6	8.2	13.1	5.35	2.14	3.66
43,944	....	2.42	29.6	8.2	18.1	5.35	1.92	3.26

Number of head.	Cost of labor per pound of gain.			Cost of feed per pound of gain.			Total cost per pound of gain.		
	High.	Low.	Average.	High.	Low.	Average.	High.	Low.	Average.
	Cents.	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*
2,068	1.95	1.43	1.67	10.37	5.81	7.84	12.32	7.24	9.51
10,360	2.09	.99	1.51	9.95	4.97	6.88	11.77	5.96	8.39
11,878	1.86	.92	1.39	8.58	4.23	6.64	10.12	5.15	8.03
15,731	2.31	.88	1.17	8.78	3.71	5.42	11.09	4.61	6.59
3,907	2.81	.98	1.73	10.39	4.17	7.28	13.14	5.15	9.01
43,944	2.81	.88	1.40	10.37	3.71	6.45	13.14	4.61	7.85

The average daily amount of grain consumed per head in the above experiment was as follows: High, 0.2593 pound; low, 0.1132 pound; average, 0.1766 pound. The total weight of the birds was 130,430 pounds.

SUMMARY OF EXPERIMENT A.

The cheapest gains and the lowest average cost of gain in this experiment was made by the lots fed 7 and 8 days. The difference in cost of gain between the 6-day lots and the 7-day lots is quite marked, while the 10-day lots show a considerably increased cost over those fed 9 days. As there was a much smaller number of birds in the 6 and 10 day lots there is a considerable possibility of error in drawing conclusions from a comparison between them and the lots fed 7, 8, and 9 days. Other conditions being equal, the lighter birds make greater gains than the heavier birds, which would partly account for the very slight difference in gain between the 8 and 9 day lots, as the average weight of the 8-day lots is much greater than the weight of the 9-day lots. This would also tend to cause the increase of the cost of gain in the 8-day lots as compared with the 7-day lots. This table shows a marked advantage in 7-day feeding over either a shorter or a longer period in producing cheap gains, but the fact that the quality of the flesh and the appearance of the bird improves with the length of the feeding period, as previously shown, should be considered in determining the best length of time to feed the birds.

EXPERIMENT B.

The lots in experiment B were fed from 6 to 15 days, the exact time depending on the season, the kind of birds, and the methods of the feeder. The feeding was carried on at station No. 1. The relative gains secured in the shorter feeding periods show distinctly that the daily gains secured in the first 6 or 7 days of the feeding period are greater than those secured on the succeeding days. The gains secured on the 7 or 8 day lots, as against the 13 or 14 day lots, show that from the standpoint of cost of gain alone the profit is much greater in the lots fed for the shorter periods. The market, however, affects to a considerable extent the best length for the feeding period, as the class into which the birds are placed when dressed depends to some extent on the length of the feeding period. Thus in order to turn out a lot as broilers it is only necessary to feed for 7 or 8 days, while if kept on feed for 13 or 14 days the same lot would be put into a different class, for which there might not be as good a demand. But allowing for all these influencing factors, the results indicate the advantages of short feeding periods with the method and under the conditions of this experiment. It is true, however, that the birds become more uniform and show the effects of milk feeding more plainly when kept on feed for the longer periods.

Stock.—This stock was very similar to that described under experiment A, but not of quite as good quality. The difference is apparent in the larger percentage of dead birds, but it is still more marked in the number of so-called "cripples." The cripples are birds off feed, which are taken from the lot and dressed during the feeding period. Their weight is credited to the dressed weight of each lot in securing both the gain in weight and the per cent gain, and it represents the difference between the gain as shown by the "weight-out" column and the number of pounds gain. The number of dead was greater in this experiment than in experiment A. In experiment A no cripples or sick birds were removed. The dead birds were picked up each day in both cases, but in experiment B special care was taken to remove all cripples as well as sick and dead birds. When sick birds occur in feeding lots this part of the work is very essential, as sickness among birds may spread rapidly where such a large number are kept together. As a general rule the amount of sickness under ordinary feeding-house conditions is small. The gains secured during the last half of September and all of October show very markedly the effect of sickness among the birds. During this period and part of November it is hard to secure good results in fattening under ordinary commercial conditions, as the chickens are apt to have colds and may develop other troubles. But the conditions under which the birds have been reared, as well as those at the feeding station, control this question.

In this experiment the lots were sorted, beginning about the middle of September, into roasters, broilers, and springs. The roasters represented the heavier chickens, the broilers the light-weight birds, with the class between termed springs. Lots which were not sorted are classed as springs in all of the records, so that this class includes many different weights of birds. While there is a great variation in the percentage of gains, the average results show that light-weight birds gain a much larger per cent than the heavier birds in the same length of time on feed; and in most cases the broilers have gained as large a per cent in 7 or 8 days as the roasters gained in 13 or 14 days. When the birds at this station were sorted, the roasters were generally fed for the longer period and the broilers for the shorter period. One reason for feeding the broilers the shorter period was because the feeder desired to turn out as many broilers as possible and still have the birds in good condition.

The breeds represented in these lots are the same as those in experiment A, and the relative proportions are quite similar. The records show that 16 per cent were Leghorns or birds belonging to the Mediterranean class, but no record was kept of the proportion of the other breeds present.

Handling.—Over three-fourths of the birds were shipped to this station in coops in live-stock cars, only a few coming in by express. Most of the birds were shipped a longer distance than those in experiment A, and many were shipped by freight although coming only a short distance. A car used entirely for poultry and eggs was switched to this station nearly every day, containing most of the eggs and poultry shipped along a branch railroad line. No live-poultry cars were received at either station No. 1 or No. 2 during the period covered by these records.

The chickens were weighed on the dock, put into a transfer battery, and carried into the feeding station, where they were placed in the stationary batteries. At the end of the feeding period the chickens were again put into the transfer batteries, weighed, and rolled into the killing room. This method of handling involved considerable labor and more handling than the method of transferring the birds described under experiment A. It was, however, preferred by the feeder of this station, who claimed that it kept the station cleaner and freer from insect pests than the other method. No trouble was apparent in station No. 3 due to the causes mentioned. The question seems to be largely one of personal preference, but the writer prefers the portable feeding battery. Birds in the stationary batteries can be fed more easily and quickly and with less spilling of feed than those in the portable batteries, as the trough in the first case is continuous, while in the latter the line is broken every 2 or 3 feet. It is also easier to scrape back feed from the longer troughs used in the stationary batteries. The stationary battery is the older and more common method of handling birds.

Feed.—Ration No. 2 was used in feeding all the lots in this experiment. This is a very good ration, and is quite similar to the one fed to the lots in experiment A except that oat flour is used in place of low-grade wheat flour and a small amount of tallow is added. The grains were mixed with ordinary buttermilk rather than condensed milk. A comparison of the results secured in these two sets of records appears to indicate that while oat flour produced as good or even better gains than wheat flour, the wheat flour gave more economical gains, and therefore was the more practical feed to use, considering the relative cost of the grains. Tallow appears to be almost too expensive to feed economically, considering the possible detrimental effect which it may have on the quality of the dressed poultry. A comparison of the results with those which follow makes one wonder how important a part the feed has in effecting the gains, as in many of those cases the cost of the gain is greater than in the present experiment, although the ration and general method of feeding were the same. Still the conditions of the feeding in experiments A and B were apparently about equal, and the conclusions drawn in a comparison of the relative value of grains should not be much out of the way if allowance is made for some differences noted elsewhere in this bulletin.

The feed was mixed by hand with a rake in portable feeding trucks (see Pl. II, fig. 1). The proportion of milk in the feed varied considerably from day to day, closely following the changes in the weather, a larger quantity being fed when the weather was hot or excessively dry. Ordinarily the amount of milk varied from 60 to 70 per cent, although in a few instances it went either much lower or much higher than these figures. The average percentages fed during certain periods were as follows: July 28 to September 9, 65.3 per cent; September 10 to October 7, 67.5 per cent; October 8 to November 6, 64 per cent; November 7 to December 5, 62.3 per cent.

The feed ordinarily appeared considerably thinner at this station than was the case in experiment A. This was due largely to the fact that low-grade wheat flour mixed with milk makes a thicker, stickier mass than an equal weight of oat flour mixed with the same amount of milk; there was, moreover, a somewhat larger percentage of milk in the ration used at this station. The birds seemed to like the thicker feed, but this principle could easily be carried to an extreme, and, as discussed previously, at least one feed daily should be relatively thin. Melted tallow was added to the mixture of grain and milk. Grit was given twice a week between regular feeding times.

Feeding.—These birds were fed twice daily, at 6.30 in the morning and between 2.30 and 4 or 5 o'clock in the afternoon, depending on the weather and on the appetite of the birds, which probably was more or less influenced by the weather. If the birds became active and restless early in the afternoon, indicating that they were hungry, the feeding was begun earlier than when they showed no desire for food until late in the day. The birds were fed only once at each feeding time, receiving a liberal feed. The feed was taken away if not cleaned up two hours after feeding in the morning but was left at night, and this feed was always cleaned up by the chickens before feeding time the next day. This method requires a more experienced feeder than the method of feeding three times daily and refeeding at each feed, as more judgment is required in regulating the amount to feed, but it gives good results if done carefully. Most beginners and inexperienced persons would get the best results by using the method described under experiment A, and on the whole it seems to be the preferable way. However, where the birds are fed only twice they are quieter during the day than if fed three times. The conditions in this feeding station tended to keep the birds quiet, as there was no confusion outside of the regular work of feeding and cleaning. No special effort was made to keep the birds in the dark. This station, like all the others, was equipped with electric lights, which were used whenever the room was dark at feeding time.

It was observed that the birds in the lower tiers invariably ate better than those in the upper tiers, probably owing to the fact that it is cooler in the lower tiers, as a large amount of heat rises from the chickens. The fact that this difference is most marked during hot weather appears to confirm this theory. The lower tiers are darker and the birds more secluded, which may also aid somewhat in fattening.

TABLE 4.—*Summary of experiment B, arranged according to length of feeding period.*

Number of head.	Days fed.	Average weight.	Per cent gain.			Grain per pound of gain.		
			High.	Low.	Average.	High.	Low.	Average.
		Pounds.	*Per cent.*	*Per cent.*	*Per cent.*	*Pounds.*	*Pounds.*	*Pounds.*
892	15	1.90	38.7	38.7	38.7	2.58	2.58	2.58
6,720	14	2.18	57.8	17.8	36.8	4.00	1.86	2.80
8,464	13	2.54	31.0	15.6	23.2	4.20	2.27	3.16
1,657	12	3.22	13.3	11.5	12.4	4.74	3.77	4.26
644	11	3.98	10.6	6.7	8.7	6.49	3.65	5.07
7,836	10	3.24	26.7	7.7	13.6	5.84	2.12	3.71
7,368	9	3.18	36.2	4.0	14.7	8.19	1.95	3.66
12,199	8	2.81	31.4	5.2	17.0	8.45	1.82	3.16
14,841	7	2.69	40.5	5.3	16.4	5.05	1.29	2.96
1,085	6	3.08	16.3	6.8	11.6	3.31	3.61	2.96
61,706	....	2.82	57.8	4.0	18.7	8.45	1.29	3.26
[1] 7,753	....	2.30	36.2	5.4	17.7	8.45	1.61	3.28
[2] 18,864	....	3.61	29.3	4.0	11.3	8.19	2.27	4.18
	6-10	....	....	....	14.7	....	....	3.49

Number of head.	Cost of labor per pound of gain.			Cost of feed per pound of gain.			Total cost per pound of gain.		
	High.	Low.	Average.	High.	Low.	Average.	High.	Low.	Average.
	Cents.	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*	*Cents.*
892	1.91	1.91	1.91	5.96	5.96	5.96	7.87	7.87	7.87
6,720	2.57	1.38	1.96	9.20	4.30	6.47	11.77	5.68	8.43
8,464	3.33	1.47	2.26	9.70	5.22	7.28	13.03	6.69	9.54
1,657	3.09	2.58	2.79	10.90	8.67	9.79	13.90	11.25	12.58
644	4.60	3.13	3.87	15.77	8.87	12.32	20.37	12.00	16.19
7,836	4.64	1.41	2.88	14.19	4.88	8.92	18.33	6.29	11.80
7,368	7.30	1.28	3.20	19.90	4.49	8.77	27.20	5.77	11.97
12,199	5.73	1.32	2.60	19.44	4.20	7.54	25.17	5.52	10.14
14,841	5.06	1.14	2.44	12.32	3.15	7.07	17.38	4.35	9.51
1,085	3.28	1.63	2.46	8.08	6.00	7.04	11.36	7.63	9.50
61,706	5.68	1.14	2.59	19.90	3.15	7.74	27.20	4.35	10.33
[1] 7,753	5.73	1.47	2.47	19.44	3.93	7.68	25.17	5.60	10.15
[2] 18,864	7.30	1.47	3.41	19.90	5.22	10.01	27.20	6.69	13.42
	....	....	2.72	....	....	7.87	....	....	10.59

[1] Broilers.

[2] Roasters.

The average daily amount of grain per head consumed in the above experiment was as follows: High, 0.2007 pound; low, 0.0758 pound; average, 0.1449 pound. The total weight of the birds was 172,792 pounds.

SUMMARY OF EXPERIMENT B.

The cheapest gain of any individual lot in this experiment was made by a 7-day lot, but the lowest average cost of gain was made by the 14 and 15 day lots. The table shows clearly that the cost of gain is in direct proportion to the average weight of the birds, which partly accounts for the cheapest gains in lots 14 and 15. The comparison of the 15-day lot with the 14-day lot is hardly fair, as only a small number of birds were fed 15 days, which was also true of the 6 and 11 day lots. The highest gain was made by a 14-day lot, which shows that very good gains can be made in the longer feeding periods. A study of the table as a whole would indicate that the results secured in 13 or 14 day feeding periods were as profitable as those obtained in the 7 and 8 day periods, allowing for the modifying conditions already noted. A comparison of the roasters and broilers shows that the broilers make the cheaper gains, which emphasizes the conclusion already drawn that the best gains are made by light birds, as all of the broilers were fed for a relatively short period, while about half of the roasters were fed for 13 or 14 days and the rest from 7 to 9 days.

COMPARISON OF EXPERIMENTS A AND B.

Comparing experiment A and experiment B it is seen that the variations in the latter are much greater than in experiment A, which shows that birds vary greatly in their ability to put on flesh, and that this variation is most marked in the longer feeding periods. It took the same average amount of grain to produce a pound of gain in both experiments, but the average total cost of producing a pound of flesh is 2.48 cents greater in experiment B than in experiment A, while the average food cost is 1.29 cents greater. Comparing lots fed from 6 up to 10 days in both experiments it is found that the average gain was 3.4 per cent greater, the grain per pound gain 0.23 pound less, the cost of labor 1.32 cents less, the cost of feed 1.42 cents less, and the total cost 2.74 cents less in experiment A than in experiment B. Therefore the ration in experiment A was a cheaper feeding ration than that used in experiment B, or, in other words, low-grade wheat flour produced cheaper gains in fattening chickens than oat flour. However, the increased amount of milk and the different methods of management undoubtedly affected the results to some extent. The cost of labor is a very important item and materially affects the cost of gain. The method of feeding in experiment A appears to be better than in experiment B, showing that feeding three times daily is better than feeding twice daily. All of these factors are closely related, which makes it impossible to determine the relative importance of each.

EXPERIMENT C.

The quality and condition of the stock in this experiment were fair, although a little lower in quality than the stock in experiments A and B. The housing and feeding conditions were slightly unfavorable during most of the period, as the birds were housed in a rented feeding station, previously described as station No. 5, until about the middle of November; after that the feeding was carried on at station No. 4. As the company intended to move their birds as soon as the new plant could be erected, no special effort was made to improve conditions in the rented feeding station; consequently much of the work was done at a disadvantage, which undoubtedly affected the results to some extent.

The lots in this experiment were fed ration No. 1, except that the proportion of corn meal and flour was varied throughout the feeding season, and that during the latter part of the period covered by this record the amount of corn meal was gradually increased about 10 per cent. A small proportion of shorts, varying from 6 to 12 per cent, with an average of 10 per cent, was added to the ration throughout the whole period. This ration was mixed with ordinary buttermilk obtained directly from a creamery, the proportion of milk in the ration varying from 58 to 65 per cent, with an average of 62 per cent for the period. These changes do not materially affect the cost of the ration.

The length of the feeding period varied from 5 to 14 days. The gains show considerable variation in the different lots, but taken as a whole the conclusions drawn from the previous records apply to the lots in this experiment. It should be stated, however, that the conditions at this station and at the station where experiment D was conducted were not studied as closely as those at the two stations represented by experiments A and B. The method of feeding was similar to that used in experiment A, except that the birds were given a very light feed at noon and not fed a second time at that meal. A tendency to leave feed in the troughs before the birds all of the time was observed. The feed was mixed by hand until the chickens were moved into the new building, where two feed mixers were installed. This feeding station (No. 4), previously described, represents the result of study and extensive experience by the manager, and contains many excellent features.

TABLE 5.—*Showing per cent gains made by chickens in experiment C, by months and by length of feeding period in each month.*

Month.	Number of head.	Days fed.	Per cent gain.		
			High.	Low.	Average.
			Per cent.	*Per cent.*	*Per cent.*
July	5,622	14	43	20	27.3
August	18,209	14	44	9	25.9
September	5,736	14	41	12	30.4
Do	1,374	13	20	10	15.0
Do	2,284	12	22	12	17.7
Do	8,477	8	28	7	15.8
Do	6,150	7	19	7	19.7
Do	6,089	6	20	2	11.9
Do	3,879	5	22	0	13.0
October	7,995	14	55	22	36.6
Do	1,765	13	41	26	32.0
Do	2,746	12	34	25	30.6
Do	2,139	11	39	31	34.0
Do	1,887	8	12	10	11.0
Do	8,854	7	16	3	9.7
Do	7,208	6	21	2	12.5
Do	3,160	5	21	1	10.5
November	1,616	14	25	11	17.8
Do	5,041	13	37	15	27.5
Do	1,175	12	27	24	25.3
Do	1,265	8	16	13	14.5
Do	6,566	7	17	8	12.6
Do	3,980	6	14	5	9.7

TABLE 6.—*Summary of experiment C, arranged according to length of feeding period.*

Number of head.	Days fed.	Per cent gain.		
		High.	Low.	Average.
		Per cent.	*Per cent.*	*Per cent.*
39,178	14	55	9	27.6
8,180	13	41	10	24.8
6,205	12	34	12	24.5
2,139	11	39	31	34.0
11,629	8	28	7	13.8
21,570	7	19	3	14.0
17,277	6	21	2	11.4
7,039	5	22	0	11.8
113,217	....	55	....	20.2

The average quantity of grain consumed daily per head in experiment C was as follows: High, 0.2396 pound; low, 0.0816 pound; average, 0.1480 pound.

EXPERIMENT D.

The stock in experiment D was of poorer quality than that at any of the other stations, and the loss from sickness and deaths during the feeding period was so large as to affect the gains adversely in most of the lots. Feeding station No. 2 was used, the equipment of which has already been described. The methods of handling and feeding and the ration used were similar to those described under experiments A and C, except that 6 per cent of shorts replaced that much corn meal until about the middle of October, when the shorts were gradually dropped from the ration. Another difference was that condensed buttermilk, diluted with water, was used in mixing the feed. The supply of milk was short during a considerable part of the feeding season and water was used freely, one feed often being mixed entirely with water. This probably partially accounts for the poorer results obtained as compared with experiment A, where, in general, the feed and methods were similar. Another factor which helps to account for the difference in results was the condition of the feeding station where the experiment was conducted. As mentioned previously in describing the house in detail, this station was very drafty in cool or cold weather, and the birds were, in consequence, subject to colds. It is impossible to tell just how much influence the condition of the house had in producing sickness, but much of it was undoubtedly caused by drafts on the birds. The station was devoted entirely to feeding, the birds being disturbed only at feeding times and when the batteries were cleaned.

A large number of chickens in these lots had their wings broken before they reached the person who graded the dressed poultry. If the wings were broken before the bird was bled, the blood would clot at the broken spot and thus injure the quality of the flesh and cause the bird to be put in a lower grade. A brief survey of the existing conditions confirmed the statement previously noted that the quality of the stock when received was poorer than that received at the other stations. These broken wings were only in rare cases noted at the other stations. The care used in handling appeared to be as good as that in either experiment A or experiment C. This condition was not extensive enough, however, to allow a selection of lots for experimental work on this point. The addition of ground bone or some kind of meat food might prevent the bones from becoming so brittle, but the principal remedy seems to lie in a change of management. The addition of bone to the ration in a few batteries did not appear to affect the gains either way.

TABLE 7.—*Per cent gains of chickens in experiment D, by months and by length of feeding period in each month.*

Month.	Number of head.	Days fed.	Per cent gain.		
			High.	Low.	Average.
			Per cent.	*Per cent.*	*Per cent.*
August	1,261	16	38	28	32.3
Do	3,091	15	39	27	34.7
Do	788	14	32	32	32.0
Do	314	8	23	12	17.5
Do	309	7	22	9	13.3
Do	1,642	6	18	6	11.9
September	1,495	16	32	28	29.7
Do	7,087	15	39	23	30.4
Do	1,952	9	16	13	14.5
Do	10,717	8	23	2	12.6
Do	3,480	7	18	9	13.3
October	3,860	15	56	22	33.2
Do	386	14	31	22	26.5
Do	3,170	9	18	11	14.3
Do	24,526	8	16	4	10.5
November	2,285	15	34	10	21.7
Do	236	14	24	24	24.0
Do	22,720	8	35	5	10.0

TABLE 8.—*Summary of experiment D, arranged according to length of feeding period.*

Number of head.	Days fed.	Per cent gain.		
		High.	Low.	Average.
		Per cent.	*Per cent.*	*Per cent.*
2,756	16	38	28	31.0
16,323	15	56	10	30.0
1,410	14	32	22	27.5
5,122	9	18	11	14.4
58,277	8	35	2	12.7
3,789	7	22	9	13.3
1,642	6	18	6	11.9
89,319	....	56	2	20.1

The average daily grain consumption per head in this experiment was as follows: High, 0.2677 pound; low, 0.0966 pound; average, 0.1754 pound.

COMPARISON OF EXPERIMENTS C AND D.

The summaries of experiments C and D confirm in general the conclusions drawn from experiments A and B in respect to the gains, but they also tend to show that the 14 and 15 day feeding periods are profitable. A comparison of the gains made in these experiments with the gains in experiment A of lots fed the same length of time shows that the gains secured in experiment A were much better, although the ration was quite similar, but the relative conditions at the stations where the experiments were conducted would largely account for this difference. This would show that the conditions at the feeding station had as much influence as the feed in producing economical gains. The records of experiments C and D also show the wide variation in results secured in feeding. The average daily grain consumption for the season in experiment D is considerably greater than in experiment C, while the average gain in these experiments is nearly equal. This difference may be partly explained by the unfavorable housing conditions and the larger per cent of deaths in experiment D. As previously stated, the station in which the birds in experiment D were fed was cold and subject to drafts in the late fall.

AVERAGE DAILY CONSUMPTION OF GRAIN PER HEAD.

Table 9 shows the average daily consumption of grain (not including milk) per bird in experiments C and D. The average grain daily in experiments A and B is found in Tables I and II of the Appendix, but in these cases the average was for the period during which the lot was on feed, and therefore does not give the daily fluctuations shown in Table 9. The milk is added in getting the cost of feed per pound of gain. This table shows how much variation there is in the amount of feed which the birds will eat from day to day. This variation is affected by the weather, by the condition of the birds, and by the method of management, as well as by the kind of food. This average daily consumption is reported to headquarters each day and serves as a close check on the success which is being obtained by the feeder, as the average gains obtained at the station vary in accordance with the amount of feed consumed.

TABLE 9.—*Average daily consumption of grain per head.*

EXPERIMENT C.

Date.	Amount consumed.	Date.	Amount consumed.	Date.	Amount consumed.	Date.	Amount consumed.
	Pounds.		*Pounds.*		*Pounds.*		*Pounds.*
August 16.....	0.1178	September 11.	0.1108	October 6.....	0.1418	October 31....	0.1774
August 17.....	.1130	September 12.	.1162	October 7.....	.1439	November 1..	.1836
August 18.....	.1380	September 13.	.1077	October 8.....	.1458	November 2..	.1949
August 19.....	.1311	September 14.	.1246	October 9.....	.1426	November 3..	.2016
August 20.....	.1326	September 15.	.1541	October 10....	.1647	November 4..	.1916
August 21.....	.1122	September 16.	.1075	October 11....	.1574	November 5..	.1883
August 22.....	.0988	September 17.	.1200	October 12....	.1532	November 6..	.1847
August 23.....	.0886	September 18.	.1080	October 13....	.1539	November 7..	.2158
August 24.....	.1138	September 19.	.1188	October 14....	.1479	November 8..	.1898
August 25.....	.0816	September 20.	.1440	October 15....	.1475	November 9..	.2120
August 26.....	.1129	September 21.	.1156	October 16....	.1612	November 10.	.1780
August 27.....	.1249	September 22.	.1067	October 17....	.1669	November 11.	.1898
August 28.....	.1250	September 23.	.0922	October 18....	.1650	November 12.	.1914
August 29.....	.1031	September 24.	.0913	October 19....	.1677	November 13.	.1718
August 30.....	.1131	September 25.	.0841	October 20....	.1646	November 14.	.2159
August 31.....	.1102	September 26.	.1033	October 21....	.1801	November 15.	.2082
September 1..	.1109	September 27.	.1266	October 22....	.1687	November 16.	.1734
September 2..	.0890	September 28.	.1381	October 23....	.1864	November 17.	.1738
September 3..	.1367	September 29.	.1329	October 24....	.1912	November 18.	.2396
September 4..	.0878	September 30.	.1255	October 25....	.1848	November 19.	.1870
September 5..	.1064	October 1.....	.1247	October 26....	.1912	November 20.	.2142
September 6..	.1025	October 2.....	.1297	October 27....	.1746	November 21.	.1689
September 7..	.1326	October 3.....	.1597	October 28....	.2035	November 22.	.1773
September 8..	.1074	October 4.....	.1576	October 29....	.1984	November 23.	.1930
September 9..	.1387	October 5.....	.1409	October 30....	.1737	November 24.	.1661
September 10.	.1125						

EXPERIMENT D.

	Pounds.		*Pounds.*		*Pounds.*		*Pounds.*
August 16.....	0.1517	September 11.	0.1220	October 6.....	0.2087	October 31....	0.2287
August 17.....	.1440	September 12.	.1755	October 7.....	.1839	November 1..	.2145
August 18.....	.1758	September 13.	.1810	October 8.....	.1918	November 2..	.1921
August 19.....	.1729	September 14.	.1543	October 9.....	.1704	November 3..	.1906
August 20.....	.2059	September 15.	.1726	October 10....	.2003	November 4..	.2233
August 21.....	.1517	September 16.	.1822	October 11....	.1561	November 5..	.1818
August 22.....	.1249	September 17.	.1804	October 12....	.1703	November 6..	.1741
August 23.....	.0982	September 18.	.1424	October 13....	.1612	November 7..	.2442
August 24.....	.0966	September 19.	.1623	October 14....	.1499	November 8..	.1830
August 25.....	.1306	September 20.	.1936	October 15....	.1674	November 9..	.1789
August 26.....	.1318	September 21.	.1328	October 16....	.1525	November 10.	.2483
August 27.....	.1373	September 22.	.1206	October 17....	.1814	November 11.	.1930
August 28.....	.1145	September 23.	.1624	October 18....	.1692	November 12.	.2012
August 29.....	.1368	September 24.	.1750	October 19....	.1553	November 13.	.2365
August 30.....	.1215	September 25.	.1103	October 20....	.1919	November 14.	.2677
August 31.....	.1604	September 26.	.1983	October 21....	.2079	November 15.	.2053
September 1..	.1183	September 27.	.1875	October 22....	.2600	November 16.	.2451
September 2..	.1383	September 28.	.1372	October 23....	.1724	November 17.	.2519
September 3..	.1207	September 29.	.1913	October 24....	.1587	November 18.	.2270
September 4..	.1185	September 30.	.1791	October 25....	.2362	November 19.	.1856
September 5..	.1862	October 1.....	.1499	October 26....	.1768	November 20.	.1962
September 6..	.1523	October 2.....	.1243	October 27....	.1522	November 21.	.2414
September 7..	.1229	October 3.....	.1624	October 28....	.2021	November 22.	.2288
September 8..	.1577	October 4.....	.1699	October 29....	.1608	November 23.	.2133
September 9..	.1532	October 5.....	.1893	October 30....	.1917	November 24.	.1965
September 10.	.1596						

DAILY DEATH RECORDS.

Table 10 gives the highest and average daily death records in percentages in each of the four experiments for the periods indicated. The remarks previously made concerning the stock under each experiment will help to explain the differences in this table. The total deaths as shown in the averages are small, but on very hot days the death record was high. October, as previously noted, is a bad month to feed on account of sickness and deaths among the birds. In experiment B the cripples were removed after the middle of September, which accounts for the low rate as compared to the other tables, where there were no cripples removed. These records confirm the statements made as to the health of the stock and the effect of the housing conditions at the various stations, allowing for the removal of the cripples in experiment B. A comparison of the death record with the percentage of gains in the respective feeding experiments shows that the death rate must be very low in order to get economical gains and that the gains vary inversely with the death rate.

TABLE 10.—*Daily death records.*

Date.	Experiment A.		Experiment B.		Experiment C.		Experiment D.	
	Highest.	Average.	Highest.	Average.	Highest.	Average.	Highest.	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
July 27–Aug. 15	1.667	0.182	0.123	0.066	....	....	....	....
Aug. 16–31	.161	.027	1.031	.166	0.262	0.108	0.222	0.132
September	.177	.038	.120	.059	.201	.039	.303	.125
October	.149	.023	.163	.061	.277	.067	.678	.210
Nov. 1–24	.463	.094	.207	.042	.373	.130	.449	.194

FATTENING HENS.

The accompanying table, No. 11, gives the results obtained in fattening a number of hens on trough feeding. Lots 1 to 6 were fed at station No. 3, and lots 7 to 14 at station No. 1. The main object of feeding was to increase the weight so as to get the hens into a higher grade, as very lightweight hens do not sell at a good price. In all these lots lightweight hens were selected and put on feed, while the heavier hens were killed without feeding any length of time. The hens to be killed without special feeding were given a full feed of corn chop and water and were held for several hours without feeding at one station before killing, but were killed with feed in their crops at another station, the food being removed after they were killed. If held without feeding and killed within a day after they reach the packing house, hens generally shrink from 1 to 3 per cent in weight. This shrinkage should be considered in calculating the profit or loss in feeding, as the hens which are fed for several days are weighed just before they are killed, while the hens which are not fed are credited with their weight as they reach the packing house.

Lots 1 to 6 were fed in various ways. The lots fed earliest in the season received cooked corn chop mixed with condensed buttermilk and water. After three or four days on this feed very bad cases of diarrhea developed, and the following lots were fed on a ration containing three-fourths corn chop and one-fourth low-grade wheat flour mixed with condensed buttermilk and water, which was scalded before being mixed with the grain. This ration kept the birds in better condition than the former feed, but did not entirely stop the diarrhea in the hens. Both the addition of low-grade flour and the scalding of the milk probably helped to stop the diarrhea. Ground bone was sprinkled lightly in the feed for a few of the batteries, and these batteries came through with little or no diarrhea when fed the ration containing the flour and scalded milk; but, as the table shows, the gains secured in all the lots were small and very variable, and the averages do not show any better gains from the second ration. Feeding the hens was then discontinued, partly because the gains cost too much and partly because there were not enough real light-weight hens to pay to feed. In selecting light hens a large number were apt to be Leghorns, which make poor gains.

Lots 7 to 14 received the same feed as the chickens in experiment B. The gains are very irregular, but a comparison with experiment B shows that it does not pay to cook the feed or make a separate mixture for the hens, as it is much easier to give the hens the same kind of feed which the chickens receive. The gains are greatly in favor of the uncooked regular feed, although most of the lots were fed longer in experiment B. A few hens off feed but not really sick may have been removed from the lots which were fed during October, so that the difference between the "Number in" and the "Number out" in these lots may not represent the dead in every case. The results at this station indicate that it pays to feed light hens, considering the increased value of the heavier birds, but apparently it is not always possible to get consistent gains. Hens do not eat their feed as quickly as springers and are harder to get on feed. If kept on feed for a long feeding period they must be watched carefully to prevent feather eating and other injurious habits. Better gains in hens are generally secured by cramming, but the labor is apt to make the total cost too high, and while some hens make very good gains when crammed, others fail to do anything.

TABLE 11.—*Results of fattening hens.*

STATION NO. 3.

Lot.	Number of birds.	Average weight in.	Dead.	Average weight out.	Dates fed.	Days fed.	Gain.	
							Pounds.	Per cent.
		Pounds.		*Pounds.*				
1	256	3.05	1	3.25	Oct. 25–Nov. 4....	11	47	6.00
2	256	2.93	3	3.13	Oct. 30–Nov. 8....	10	41	5.50
3	256	3.01	3	3.28	Oct. 13–19........	7	59	7.70
4	320	2.88	2	3.08	Oct. 8–14.........	7	58	6.30
5	320	2.91	4	3.03	Nov. 2–6..........	5	24	2.60
6	256	2.98	0	3.10	Oct. 17–21........	5	32	4.20

TABLE 11.—*Results of fattening hens*—Continued.

STATION NO. 1.

Lot.	Number of birds.	Average weight in.	Number out.[1]	Average weight out.	Dates fed.	Days fed.	Gain.	
							Pounds.	Per cent.
		Pounds.		*Pounds.*				
7	340	3.22	316	3.47	Sept. 29–Oct. 9...	11	32	2.00
8	340	3.46	336	4.06	Aug. 30–Sept. 9...	11	190	16.20
9	340	2.84	309	3.48	Oct. 2–12.........	11	207	21.00
10	340	3.59	314	3.68	Oct. 23–Nov. 1....	10	57	4.00
11	340	2.72	318	3.45	Oct. 30–Nov. 8....	10	241	26.00
12	340	3.37	309	3.74	Aug. 21–28........	8	9	.80
13	680	3.13	627	3.37	Oct. 13–19........	7	231	10.00
14	340	2.94	301	3.85	Oct. 17–23........	7	288	28.00

[1] Some hens were removed from these lots while on feed, so that the difference between the number in and the number out does not always represent dead birds.

SHRINKAGE IN DRESSING.

The birds were dressed and put into cold storage. The loss of weight or shrinkage in dressing (without drawing) for the different classes of birds varied as follows: Hens, 13.4 to 14.9 per cent, average 14.4 per cent; roasters, 13.7 to 16 per cent, average 14.7 per cent; springs, 9 to 14.5 per cent, average 12.1 per cent; and broilers, 14 to 14.7 per cent, average 14.3 per cent. The chickens at the different stations were fed a mixture of fine sand and very thin feed, or were first given a light feed and then sand and water for the last feed of the day before they were killed. Several of the pickers claimed that the chickens picked easier if the birds were watered freely before killing, and this practice was prevalent, although there was some difference of opinion as to the effect of the water.

CLEANING AND SPRAYING THE BATTERIES.

Where chickens are kept in large numbers cleanliness is a very important factor. Great care was exercised at all of the feeding stations to keep the batteries clean. In some stations the batteries were cleaned every day, in others, every other day, the latter plan prevailing at the majority of the stations. The droppings were scraped from the trays with a tin scraper. A scraper a trifle over half the width of the tray saves time in cleaning, if holding it does not tire the operator too much. Some prefer a narrower scraper. Many feeders dust air-slaked lime on each tray after cleaning. Another good method, which takes less time, is to spray the tray lightly with a hand sprayer after it is put back into the battery, using a coal-tar disinfectant. The batteries are generally sprayed with whitewash after each lot has been removed, although sometimes this was neglected and the batteries were only sprayed about once a month. The frequency of cleaning depends somewhat on the length of the feeding period, but batteries should be sprayed at least twice a month. Lime keeps the insect pests away.

POULTRY MANURE.

A large amount of poultry manure is produced daily at the feeding stations, and this has considerable fertilizing value, but most of the managers find it a source of extra expense instead of revenue. At one station the droppings were washed through a sewer into a river, which involved considerable labor; at other places the manure was loaded into an empty wagon kept for that purpose, which was hauled away each day. In some localities in the Middle West the packers can get farmers to remove the manure without paying for the labor of hauling, but as the feeding stations are usually located in the towns or cities the manure must be removed regularly and promptly, or it becomes a public nuisance. In such cases the manure should be taken away daily, and farmers are apt to be irregular about doing the work. Various methods of drying the droppings and putting them on the market for fertilizer have been suggested and tried on a small scale, but apparently without success. The farmers in this section will learn the value and necessity of using manure on their land in time, and will then be glad to avail themselves of a daily supply of poultry droppings for nothing.

Some of the packers hope to operate farms in connection with their feeding stations, thus utilizing the manure to good advantage. Rightly handled, a farm should be a profitable undertaking in connection with a feeding station, not only utilizing the various byproducts, but also as a breeding establishment for poultry, where the packer could raise purebred stock of the best market types to distribute in the territory from which he draws his products. This distribution could be done either at a nominal price or in exchange for the same weight of undesirable types of male birds of mixed breeding. The packer could also select the best pullets from the stock shipped in to be marketed and keep them for spring egg production, killing these pullets at the end of their laying period, when they would be worth about as much for dressed poultry as they had cost in the fall or winter.

Records of the amount of droppings from the fattening stock kept at various times during the feeding season showed that the number of pounds produced per 100 birds varied from day to day. One hundred "springs" averaged 11 pounds of manure daily, which would mean over half a ton of manure a day for each 10,000 chickens on feed. The consistency of the droppings is a fair indication of the condition of the bowels of the chickens. The droppings should be soft but not watery. Confinement and sour milk make softer droppings than are obtained from poultry kept on the range and fed on whole or ground grains. The feeder should observe the droppings occasionally and feed accordingly.

In case the birds have excessive diarrhea, it is a good plan to scald the milk for one or two feedings, which will generally bring the birds back to normal condition.

KEEPING RECORDS.

Careful records were kept of all the operations in the packing houses, for which purpose various systems of checking and rechecking the different lots of chickens were used. The person in charge of each room or branch of the work made reports to the office covering the work done in his room for each day. The packers, of course, aim to systematize labor, save any wastes for by-products, and reduce cost in every line, at the same time improving the quality of the product. A good type of feeding-station report is shown below. Careful records were kept of the cost of producing gains and of killing and dressing birds, while some of the packers had elaborate records which showed all the expenses incurred by a lot of chickens until they went into storage. By a careful study of these records the manager of the packing house could tell what his product was costing, and could figure out how the cost was divided. There are many different ideas as to the best way to keep such records, but the object should be to show the cost of each part of the work accurately, yet as simply as possible.

FEEDING-STATION REPORT.

Date......................., 191 .

Total number of chickens on feed to-day............

FEED.

Fed to-day.	Weight.	Price.	Total cost.	Weight fed per 100 head.
Corn meal.......pounds..	....	....	....	....
Oatmeal.......do....	....	....	....	....
Milk.......gallons..	....	....	....	....
Tallow.......pounds..	....	....	....	....
Meat.......do....	....	....	....	....
Grit..........	....	....	....	....
Total..........	....	....	....	....

COST OF FEED PER 100 HEAD.

Cost of feed per 100 head..........	$........
Cost of labor per 100 head..........	....
Total average cost per 100 head..........	....
Total cost labor to-day..........	....

CHICKENS PUT ON FEED.

	Head.	Weight.	Average weight.
	Number.	*Pounds.*	*Pounds.*
Put on feed to-day			
Put on feed to-day			
Put on feed to-day			
Put on feed to-day			

CHICKENS KILLED.

	Days fed.	Head.	Weight.	Gain.	Per cent gain.	Died.
		Number.	*Pounds.*	*Pounds.*	*Per cent.*	*Number.*
Killed to-day						
Killed to-day						
Killed to-day						
Killed to-day						

Sick to-day..............head. Weight..............pounds. Average weight.........pounds.
Dead to-day..............head. Weight..............pounds. Average weight.........pounds.

(Signed) ------------------------------, *Manager.*

CONCLUSIONS.

1. The Plymouth Rock and other varieties of general-purpose fowls make more economical gains in fattening than the Mediterranean class, such as Leghorns.

2. Chickens of the same breed vary greatly in their ability to put on flesh. This variation may lead to gross error in drawing conclusions from experiments in feeding poultry which deal with only a small number of birds.

3. Muslin or duck cloth can be used to good advantage to replace the windows or part of the walls of feeding stations.

4. If a feeding station is properly constructed, good ventilation can be secured without having a large open space in the top of the building, such as a monitor top. Such buildings can be constructed more cheaply than those with a large amount of air space per bird, by using muslin curtains for the walls.

5. The use of portable feeding batteries is more easily adapted to varying conditions, involves less labor, and turns the birds out in better condition than the stationary batteries.

6. Low-grade wheat flour is a more economical feed than oat flour in fattening rations for chickens at the present prices of grain.

7. The average person will get better results in fattening by feeding three times rather than twice daily.

8. The amount of grain required to produce a pound of flesh in fattening chickens varied in experiment A from 1.92 to 5.35 pounds, with an average of 3.26 pounds; while in experiment B the amount varied from 1.29 to 8.45 pounds, with an average of 3.26 pounds.

The total cost of feed per pound of gain varied from 3.71 to 10.37 cents, and averaged 6.45 cents in experiment A, while in experiment B the cost varied from 3.15 to 19.90 cents, and averaged 7.74 cents.

The cost of labor for a pound of gain in flesh varied from 0.88 to 2.81 cents and averaged 1.40 cents in experiment A, while in experiment B the cost varied from 1.14 to 5.63 cents, and averaged 2.59 cents.

The cost of both feed and labor to produce a pound of gain in fattening varied from 4.61 to 13.14 cents, and averaged 7.85 cents in experiment A; and it varied from 4.35 to 27.20 cents, and averaged 10.33 cents in experiment B.

The average total cost of feed and labor per pound of gain for all the birds in experiments A and B was 9.09 cents; the average cost of feed alone, 7.10 cents.

9. The cheaper gains were made in the shorter feeding periods (7 or 8 days) and by the light chickens.

10. Hens make poorer gains than chickens in crate fattening. Fattening hens by this method is profitable only under certain conditions.

APPENDIX.

TABLE I.—*Details of feeding experiment A.*

Lot.	Class.	Number in.	Total weight.	Average weight.	Dates fed.	Days fed.	Total feed.	Number out.[1]	Total weight.	Average weight.	Total gain.	Per cent gain.	Average number fed.[2]	Average grain daily per head.	Grain per pound of gain.	Total cost of labor.	Cost of labor per pound of gain.	Cost of feed per pound of gain.[3]	Total cost per pound of gain.
		Head.	*Pounds.*	*Pounds.*			*Pounds.*	*Head.*	*Pounds.*	*Pounds.*	*Pounds.*	*Per ct.*	*Head.*	*Pounds.*	*Pounds.*	*Dollars.*	*Cents.*	*Cents.*	*Cents.*
1	Springers.	480	900	1.88	Aug. 6 to 15...	10	706.0	477	1,093	2.29	193	21.4	478.5	0.1476	3.66	3.49	1.81	7.06	8.87
2	...do......	240	481	2.00	Aug. 7 to 15....	9	317.0	238	556	2.34	75	15.6	239.0	.1476	4.23	1.57	2.09	8.16	10.25
3	...do......	160	312	1.95	Aug. 10 to 15..	6	138.0	159	341	2.14	29	9.3	159.5	.1442	4.76	.70	2.41	9.19	11.60
4	...do......	400	771	1.93	Aug. 10 to 16..	7	400.0	395	859	2.17	88	11.4	397.5	.1436	4.55	2.03	2.31	8.78	11.09
5	...do......	640	1,212	1.89	Aug. 12 to 17..	6	530.0	633	1,311	2.07	99	8.2	636.5	.1389	5.35	2.78	2.81	10.33	13.14
6	...do......	240	435	1.81	Aug. 14 to 22..	9	321.0	239	541	2.26	106	24.4	239.5	.1491	3.03	1.57	1.48	5.85	7.33
7	...do......	320	620	1.94	Aug. 16 to 23..	8	377.0	318	733	2.31	113	18.2	319.0	.1478	3.34	1.86	1.65	6.45	8.10
8	...do......	480	972	2.03	Aug. 17 to 24..	8	566.0	479	1,147	2.39	175	18.0	479.5	.1475	3.23	2.80	1.60	6.23	7.83
9	...do......	160	308	1.93	Aug. 18 to 25..	8	191.0	159	358	2.25	50	16.2	159.5	.1498	3.82	.93	1.86	7.37	9.23
10	...do......	240	520	2.17	Aug. 19 to 28..	10	367.0	239	642	2.69	122	23.5	239.5	.1532	3.01	1.75	1.43	5.81	7.24
11	...do......	640	1,224	1.91	Aug. 20 to 28..	9	876.0	638	1,469	2.30	245	20.0	639.0	.1524	3.58	4.19	1.71	6.91	8.62
12	...do......	480	910	1.90	Aug. 21 to 29..	9	630.0	480	1,140	2.38	230	25.3	480.0	.1458	2.74	3.15	1.37	5.29	6.66
13	...do......	400	756	1.89	Aug. 23 to 31..	9	521.0	398	928	2.33	172	22.8	399.0	.1452	3.03	2.62	1.52	5.85	7.37
14	...do......	320	714	2.23	Aug. 24 to Sept. 1.	9	440.0	319	875	2.74	161	22.5	319.5	.1530	2.73	2.10	1.30	5.27	6.57
15	...do......	160	307	1.92	Aug. 26 to Sept. 1.	7	175.0	160	398	2.49	91	29.6	160.0	.1560	1.92	.82	.90	3.71	4.61
16	...do......	800	1,646	2.06	Aug. 27 to Sept. 2.	7	883.0	798	1,939	2.43	293	17.8	799.0	.1578	3.01	4.08	1.39	5.81	7.20
17	...do......	400	785	1.96	Aug. 28 to Sept. 5.	9	558.0	397	968	2.44	183	23.3	398.5	.1556	3.05	2.61	1.43	5.89	7.32
18	...do......	240	526	2.19	Aug. 30 to Sept. 6..	8	296.0	237	618	2.61	92	17.5	238.5	.1551	3.22	1.39	1.51	6.21	7.72
19	...do......	720	1,575	2.19	Aug. 31 to Sept. 6.	7	794.0	717	1,916	2.67	341	21.7	718.5	.1578	2.33	3.67	1.08	4.50	5.58
20	...do......	1,440	3,205	2.23	Sept. 1 to 7...	7	1,606.0	1,436	3,746	2.61	541	16.9	1,438.0	.1596	2.97	7.34	1.36	5.73	7.09
21	...do......	800	1,865	2.33	Sept. 1 to 9...	9	1,110.0	794	2,161	2.72	296	15.9	797.0	.1548	3.75	5.23	1.77	7.73	9.50
22	...do......	480	1,006	2.10	Sept. 2 to 9...	8	580.0	478	1,230	2.57	224	22.3	479.0	.1513	2.59	2.79	1.25	5.34	6.59

[1] The difference between the "Number in" and the "Number out" represents the dead birds in this table. Such is not the case with Table II, where the "cripples" were removed during the feeding period and their dressed weight credited to the lot when it was killed.

[2] For the purpose of computing results, the "Average number fed" is arrived at by adding the "Number in" and the "Number out" and dividing by 2, thus getting an average.

[3] The cost of feed per pound of gain includes the cost of the milk, which is added to the cost of the grain.

TABLE I.—*Details of feeding experiment A*—Continued.

Lot.	Class.	Number in.	Total weight.	Average weight.	Dates fed.	Days fed.	Total feed.	Number out.	Total weight	Average weight.	Total gain.	Per cent gain.	Average number fed.	Average grain daily per head.	Grain per pound of gain.	Total cost of labor.	Cost of labor per pound of gain.	Cost of feed per pound of gain.	Total cost per pound of gain.
		Head.	*Pounds.*	*Pounds.*			*Pounds.*	*Head.*	*Pounds.*	*Pounds.*	*Pounds.*	*Per ct.*	*Head.*	*Pounds.*	*Pounds.*	*Dollars.*	*Cents.*	*Cents.*	*Cents.*
23	Springers.	1,200	2,686	2.24	Sept. 7 to 13..	7	1,391.0	1,200	3,156	2.63	470	17.5	1,200.0	0.1656	2.96	6.12	1.30	6.10	7.40
24	...do......	480	1,049	2.19	Sept. 8 to 15..	8	648.0	478	1,236	2.59	187	17.8	479.0	.1692	3.47	2.79	1.49	7.15	8.64
25	...do......	720	1,681	2.33	Sept. 9 to 15..	7	877.0	718	2,012	2.80	331	19.7	719.0	.1743	2.65	3.67	1.11	5.46	6.57
26	...do......	720	1,693	2.35	Sept. 10 to 16..	7	910.0	719	2,024	2.82	331	19.6	719.5	.1806	2.75	3.67	1.11	5.67	6.78
27	...do......	640	1,525	2.38	Sept. 11 to 18..	8	875.0	635	1,776	2.80	251	16.5	637.5	.1715	3.49	3.72	1.48	7.19	8.67
28	...do......	480	1,101	2.29	Sept. 13 to 18..	6	500.0	479	1,220	2.55	119	10.8	479.5	.1739	4.20	2.10	1.76	8.65	10.41
29	...do......	1,040	2,396	2.30	Sept. 14 to 19..	6	1,060.0	1,029	2,779	2.70	383	16.0	1,034.5	.1708	2.77	4.52	1.18	5.71	6.89
30	...do......	960	2,163	2.25	Sept. 16 to 21..	6	940.0	958	2,506	2.62	343	15.9	959.0	.1634	2.74	4.19	1.22	5.64	6.86
31	...do......	1,200	2,848	2.37	Sept. 17 to 23..	7	1,291.0	1,194	3,375	2.83	527	18.5	1,197.0	.1541	2.45	6.11	1.16	5.05	6.21
32	...do......	880	2,049	2.33	Sept. 18 to 25..	8	1,071.0	875	2,387	2.73	338	16.5	877.5	.1526	3.17	5.12	1.51	6.53	8.04
33	...do......	400	960	2.40	Sept. 20 to 26..	7	442.0	400	1,139	2.85	179	18.6	400.0	.1578	2.47	2.04	1.14	5.09	6.23
34	...do......	400	886	2.22	Sept. 19 to 25..	7	431.0	398	1,096	2.75	210	23.7	399.0	.1544	2.05	2.04	.97	4.22	5.19
35	...do......	1,600	3,778	2.36	Sept. 22 to 28..	7	1,817.0	1,592	4,623	2.90	845	22.4	1,596.0	.1626	2.15	8.14	.96	4.43	5.39
36	...do......	480	1,204	2.51	Sept. 23 to 29..	7	550.0	477	1,414	2.96	210	17.4	478.5	.1688	2.62	2.44	1.16	5.40	6.56
37	...do......	640	1,488	2.33	Sept. 24 to Oct. 2.	9	958.0	639	1,856	2.90	368	24.7	639.5	.1664	2.60	4.20	1.14	5.36	6.50
38	...do......	1,040	2,729	2.62	Sept. 25 to Oct. 3.	9	1,533.0	1,032	3,172	3.07	443	16.2	1,036.0	.1644	3.46	6.80	1.53	6.75	8.28
39	...do......	640	1,533	2.40	Sept. 28 to Oct. 3.	6	610.0	636	1,818	2.86	285	18.6	638.0	.1594	2.14	2.79	.98	4.17	5.15
40	...do......	800	2,067	2.58	Sept. 29 to Oct. 5.	7	927.0	792	2,530	3.19	463	23.6	796.0	.1662	2.00	4.06	.88	3.90	4.78
41	...do......	1,360	3,198	2.35	Sept. 29 to Oct. 6.	8	1,880.0	1,358	4,064	2.99	866	27.1	1,359.0	.1726	2.17	7.98	.92	4.23	5.15
42	...do......	800	2,018	2.52	Oct. 2 to 10....	9	1,342.0	792	2,545	3.21	527	26.1	796.0	.1873	2.55	5.22	.99	4.97	5.96
43	...do......	800	2,068	2.59	Oct. 5 to 11....	7	1,107.0	798	2,388	2.99	320	15.5	799.0	.1980	2.46	4.08	1.28	6.75	8.03
44	...do......	800	2,104	2.63	Oct. 6 to 12....	7	1,073.0	797	2,522	3.16	418	19.9	798.5	.1921	2.57	4.07	.97	5.01	5.98
45	...do......	480	1,265	2.64	Oct. 7 to 13....	7	645.0	480	1,497	3.12	232	18.3	480.0	.1918	2.78	2.45	1.06	5.42	6.48
46	...do......	720	1,958	2.72	Oct. 9 to 17....	9	1,249.0	716	2,370	3.31	412	21.0	718.0	.1932	3.03	4.71	1.14	5.91	7.05
47	...do......	560	1,543	2.76	Oct. 11 to 18...	8	857.0	560	1,793	3.20	250	16.2	560.0	.1912	3.43	3.27	1.31	6.69	8.00
48	...do......	640	1,680	2.62	Oct. 12 to 18...	7	873.0	639	2,001	3.13	321	19.1	639.5	.1949	2.72	3.26	1.02	5.30	6.32
49	...do......	1,200	3,406	2.84	Oct. 14 to 20...	7	1,671.0	1,195	4,049	3.39	643	18.9	1,197.5	.1993	2.60	6.11	.95	5.07	6.02
50	...do......	800	2,197	2.75	Oct. 15 to 21...	7	1,152.0	797	2,514	3.15	317	14.4	798.5	.2060	3.63	4.07	1.28	7.08	8.36
51	...do......	880	2,429	2.76	Oct. 16 to 23...	8	1,454.0	874	2,938	3.36	509	20.5	877.0	.2073	2.86	5.11	1.00	5.58	6.58
52	...do......	960	2,991	3.12	Oct. 19 to 26...	8	1,622.0	955	3,440	3.60	449	15.0	957.5	.2117	3.61	5.58	1.24	7.04	8.28
53	...do......	640	1,945	3.04	Oct. 25 to Nov. 1.	8	1,036.0	639	2,298	3.60	353	18.1	639.5	.2024	2.93	3.78	1.06	5.71	6.77

54	...do......	800	2,179	2.72	Oct. 26 to Nov. 2.	8	1,279.0	797	2,504	3.14	325	14.9	798.5	.2001	3.94	4.66	1.43	7.68	9.11
55	...do......	720	2,322	3.23	Oct. 30 to Nov. 8.	10	1,425.0	713	2,590	3.63	268	11.5	716.5	.1968	5.32	5.22	1.95	10.37	12.32
56	...do......	640	1,892	2.96	Oct. 30 to Nov. 7.	9	1,149.0	634	2,216	3.50	324	17.1	637.0	.2004	3.55	4.18	1.29	6.92	8.21
57	...do......	640	1,997	3.12	Nov. 1 to 8....	8	1,009.0	632	2,293	3.63	296	14.8	636.0	.1982	3.41	3.71	1.25	6.65	7.90
58	...do......	640	1,774	2.77	Nov. 4 to 13...	10	1,293.0	627	2,085	3.33	311	17.6	633.5	.2040	4.16	4.62	1.49	8.11	9.60
59	...do......	1,200	3,807	3.17	Nov. 6 to 14...	9	2,161.0	1,188	4,246	3.57	439	11.5	1,194.0	.2001	4.92	7.83	1.78	9.59	11.37
60	...do......	880	2,611	2.97	Nov. 3 to 11...	9	1,601.0	863	2,925	3.39	314	12.0	871.5	.2041	5.10	5.72	1.82	9.95	11.77
61	...do......	960	2,960	3.08	Nov. 10 to 17..	8	1,584.0	941	3,320	3.53	360	12.2	950.5	.2082	4.40	5.54	1.54	8.58	10.12
62	...do......	1,440	4,873	3.38	Nov. 11 to 18..	8	2,275.0	1,422	5,406	3.80	533	10.9	1,431.0	.1987	4.27	8.35	1.57	8.33	9.90
63	...do......	960	3,001	3.13	Nov. 13 to 21..	9	1,683.0	953	3,338	3.50	337	11.2	956.5	.1955	4.99	6.28	1.86	9.73	11.59

TABLE II.—*Details of feeding experiment B.*

Lot.	Class.	Number in.	Total weight.	Average weight.	Dates fed.	Days fed.	Total feed.	Number out.[1]	Total weight.	Average weight.	Total gain.	Per cent gain.	Average number fed.[2]	Dead.	Average grain daily per head.	Grain per pound of gain.	Total cost of labor.	Cost of labor per pound of gain.	Cost of feed per pound of gain.	Total cost per pound of gain.
		Head.	*Pounds.*	*Pounds.*			*Pounds.*	*Head.*	*Pounds.*	*Pounds.*	*Lbs.*	*Per ct.*	*Head.*		*Pounds.*	*Pounds.*	*Dollars.*	*Cents.*	*Cents.*	*Cents.*
1	Springers.	450	835	1.86	July 28 to Aug. 10.	14	865.3	442	1,154	2.61	319	38.2	446.0	5	0.1386	2.71	5.80	1.84	6.26	8.10
2	...do......	450	858	1.91	July 29 to Aug. 11.	14	819.5	435	1,164	2.68	306	35.7	442.5	3	.1338	2.68	5.85	1.91	6.19	8.10
3	...do......	450	744	1.65	July 30 to Aug. 12.	14	799.8	449	1,174	2.61	430	57.8	449.5	1	.1271	1.86	5.94	1.38	4.30	5.68
4	...do......	450	817	1.82	Aug. 3 to 16...	14	804.7	444	1,099	2.48	282	34.5	447.0	6	.1286	2.85	5.91	2.10	6.58	8.68
5	...do......	1,130	2,300	2.04	Aug. 4 to 17...	14	2,045.0	1,119	3,084	2.76	784	34.1	1,124.5	11	.1299	2.61	14.86	1.90	6.03	7.93
6	...do......	1,125	2,186	1.94	Aug. 5 to 18...	14	2,024.0	1,120	3,008	2.69	822	37.6	1,122.5	11	.1288	2.46	14.83	1.80	5.68	7.48
7	...do......	1,350	2,588	1.92	Aug. 6 to 19...	14	2,455.0	1,348	3,617	2.68	1,029	39.8	1,349.0	8	.1300	2.39	17.83	1.73	5.52	7.25
8	...do......	900	1,712	1.90	Aug. 7 to 21...	15	1,713.0	885	2,375	2.68	663	38.7	892.5	14	.1280	2.58	12.64	1.91	5.96	7.87
9	...do......	1,125	2,029	1.80	Aug. 8 to 15...	8	1,159.0	1,107	2,668	2.41	637	31.4	1,116.0	15	.1298	1.82	8.43	1.32	4.20	5.52
10	...do......	1,125	2,325	2.07	Aug. 11 to 23..	13	1,769.0	1,110	3,046	2.74	721	31.0	1,117.5	14	.1218	2.45	13.71	1.90	5.66	7.56
11	...do......	1,125	2,315	2.06	Aug. 12 to 24..	13	1,727.0	1,110	2,933	2.64	618	26.7	1,117.5	21	.1189	2.79	13.71	2.22	6.44	8.66
12	...do......	1,125	2,319	2.06	Aug. 13 to 25..	13	1,707.0	1,110	2,988	2.69	669	29.8	1,117.5	6	.1175	2.55	13.71	2.05	5.89	7.94
13	...do......	1,125	2,398	2.13	Aug. 14 to 26..	13	1,687.0	1,054	2,800	2.66	402	16.8	1,089.5	40	.1191	4.20	13.37	3.33	9.70	13.03
14	...do......	1,125	2,499	2.22	Aug. 16 to 22..	7	936.0	1,094	2,696	2.46	197	7.9	1,109.5	6	.1205	4.75	7.33	3.72	10.97	14.69
15	...do......	1,125	2,466	2.19	Aug. 19 to 28..	10	1,323.0	1,110	2,992	2.70	526	21.3	1,117.5	16	.1184	2.52	10.55	2.01	5.82	7.83
16	...do......	1,125	2,554	2.27	Aug. 21 to 29..	9	1,211.0	1,112	3,137	2.82	583	22.8	1,118.5	13	.1203	2.08	9.50	1.63	4.80	6.43
17	...do......	1,125	2,517	2.24	Aug. 22 to 30..	9	1,246.0	1,114	3,121	2.80	604	24.0	1,119.5	11	.1237	2.06	9.51	1.57	4.76	6.33
18	...do......	1,125	2,447	2.18	Aug. 24 to 31..	8	1,192.0	1,116	3,065	2.75	618	25.3	1,120.5	7	.1333	1.93	8.46	1.37	4.46	5.83
19	...do......	1,125	2,629	2.34	Sept. 2 to 9....	8	1,322.0	1,130	3,305	2.92	541	20.6	1,127.5	2	.1466	2.44	8.51	1.57	5.64	7.21
20	...do......	1,350	3,607	2.67	Sept. 9 to 15...	7	1,332.0	1,354	4,251	3.14	644	17.9	1,352.0	12	.1406	2.07	9.02	1.40	4.76	6.16
21	...do......	1,350	3,416	2.53	Sept. 10 to 16..	7	1,391.0	1,353	4,210	3.11	793	23.2	1,351.5	12	.1467	1.75	9.02	1.14	4.03	5.17
22	...do......	1,350	3,509	2.60	Sept. 11 to 18..	8	1,599.0	1,341	4,194	3.13	685	19.5	1,345.5	9	.1485	2.33	10.26	1.48	5.36	6.84
23	...do......	1,350	3,400	2.52	Sept. 12 to 21..	10	1,924.0	1,340	4,307	3.21	907	26.7	1,345.0	10	.1430	2.12	12.82	1.41	4.88	6.29
24	...do......	1,350	3,666	2.72	Sept. 13 to 22..	10	1,916.0	1,338	4,183	3.13	517	14.1	1,344.0	12	.1425	3.71	12.81	2.48	8.53	11.01
25	Broilers...	450	820	1.82	Sept. 13 to 21..	9	580.0	446	1,217	2.73	297	36.2	448.0	4	.1439	1.95	3.84	1.28	4.49	5.77
26	Roasters..	1,350	4,031	2.99	Sept. 16 to 28..	13	2,600.0	1,336	4,827	3.62	796	19.7	1,343.0	14	.1489	3.27	16.64	2.09	7.52	9.61
27	Broilers...	900	2,204	2.45	Sept. 16 to 23..	8	1,006.0	890	2,325	2.61	119	5.4	895.0	9	.1405	8.45	6.82	5.73	19.44	25.17
28	Roasters..	900	2,806	3.12	Sept. 17 to 29..	13	1,718.0	885	3,243	3.66	437	15.6	892.5	10	.1480	3.93	11.06	2.53	9.04	11.57
29	Broilers...	450	1,069	2.38	Sept. 17 to 23..	7	453.0	452	1,174	2.60	106	9.9	451.0	1	.1370	4.08	3.01	2.84	9.38	12.22
30	Roasters..	900	2,794	3.10	Sept. 19 to Oct. 2.	14	1,825.0	889	3,291	3.70	497	17.8	894.5	11	.1457	3.67	11.93	2.40	8.44	10.84
31	Broilers...	450	1,065	2.37	Sept. 20 to 26..	7	453.0	449	1,228	2.73	163	15.3	449.5	1	.1438	2.78	3.00	1.84	6.39	8.23
32	Roasters..	900	2,755	3.06	Sept. 21 to Oct. 3.	13	1,726.0	894	3,207	3.59	452	16.4	897.0	6	.1480	3.82	11.11	2.46	8.79	11.25

33	Broilers...	900	2,003	2.23	Sept. 22 to 28..	7	975.0	893	2,210	2.47	207	10.3	896.5	5	.1553	4.71	5.98	2.89	10.83	13.72
34	Roasters..	450	1,510	3.36	Sept. 22 to Oct. 5.	14	925.0	440	1,721	3.91	231	15.3	445.0	3	.1485	4.00	5.94	2.57	9.20	11.77
35	...do......	900	2,954	3.28	Sept. 24 to Oct. 5.	12	1,615.0	891	3,285	3.69	341	11.5	895.5	5	.1503	4.74	10.24	3.00	10.90	13.90
36	Broilers...	450	960	2.13	Sept. 25 to Oct. 2.	8	555.0	449	1,196	2.66	233	24.3	449.5	1	.1542	2.38	3.43	1.47	5.47	6.94
37	Roasters..	900	2,555	2.84	Sept. 26 to Oct. 8.	13	1,698.0	880	3,278	3.73	749	29.3	890.0	15	.1468	2.27	11.03	1.47	5.22	6.69
38	Broilers...	450	963	2.14	Sept. 27 to Oct. 2.	6	409.0	446	1,120	2.51	157	16.3	448.0	8	.1522	2.61	2.56	1.63	6.00	7.63
39	Roasters..	800	2,525	3.16	Sept. 30 to Oct. 11.	12	1,270.0	724	2,674	3.69	337	13.3	762.0	7	.1389	3.77	8.71	2.58	8.67	11.25
40	...do......	800	2,742	3.43	Oct. 2 to 10....	9	1,005.0	747	2,858	3.83	235	8.6	773.5	0	.1856	4.28	6.63	2.82	9.84	12.66
41	Broilers...	900	2,084	2.32	Oct. 2 to 8.....	7	885.0	869	2,277	2.62	235	11.3	884.5	0	.1430	3.77	5.90	2.51	8.67	11.18
42	Roasters..	800	2,747	3.43	Oct. 5 to 13....	9	966.0	784	2,876	3.67	314	11.4	792.0	3	.1355	3.08	8.60	2.74	7.48	10.22
43	...do......	400	1,390	3.48	Oct. 6 to 14....	9	457.0	360	1,312	3.64	105	7.6	380.0	1	.1337	4.35	4.12	3.92	10.57	14.49
44	Springers .	680	2,113	3.11	Oct. 7 to 16....	10	887.0	636	2,339	3.68	248	11.7	658.0	3	.1348	3.58	7.94	3.20	8.70	11.90
45	Broilers...	450	994	2.21	Oct. 8 to 14	7	410.0	429	1,105	2.58	157	15.8	439.5	2	.1333	2.61	3.71	2.36	6.34	8.70
46	Roasters ..	800	2,919	3.65	Oct. 9 to 17 ...	9	958.0	774	2,965	3.83	117	4.0	787.0	3	.1352	8.19	8.54	7.30	19.90	27.20
47	Broilers...	450	1,168	2.60	Oct. 9 to 15	7	419.0	431	1,217	2.82	90	7.7	440.5	2	.1358	4.66	3.72	4.13	11.32	15.45
48	Springers .	400	1,073	2.68	Oct. 12 to 18...	7	366.0	387	1,481	3.83	144	13.4	393.5	1	.1329	2.54	3.32	2.31	6.17	8.48
49	...do......	336	1,283	3.82	Oct. 13 to 23...	11	497.0	304	1,341	4.41	136	10.6	320.0	3	.1412	3.65	4.25	3.13	8.87	12.00
50	...do......	400	1,275	3.19	Oct. 14 to 23...	10	542.0	357	1,310	3.67	169	13.3	378.5	6	.1432	3.21	4.56	2.70	7.80	10.50
51	...do......	400	1,334	3.34	Oct. 15 to 24...	10	578.0	378	1,384	3.66	147	11.0	389.0	2	.1485	3.93	4.69	3.19	9.55	12.74
52	...do......	800	2,482	3.10	Oct. 18 to 25...	8	945.0	723	2,620	3.62	317	12.8	761.5	3	.1550	2.98	7.35	2.32	7.24	9.56
53	...do......	800	2,652	3.32	Oct. 19 to 26...	8	991.0	769	2,848	3.70	272	10.3	784.5	0	.1579	3.64	7.57	2.78	8.85	11.63
54	...do......	800	2,230	2.79	Oct. 21 to 27...	7	901.0	749	2,424	3.24	358	16.1	774.5	6	.1662	2.52	6.42	1.79	6.12	7.91
55	Roasters ..	340	1,050	3.09	Oct. 21 to 30...	10	543.0	312	1,399	4.48	141	13.4	326.0	2	.1666	3.85	3.93	2.79	9.36	12.15
56	Springers .	400	1,129	2.82	Oct. 24 to 30...	7	466.0	370	1,205	3.26	187	16.6	385.0	0	.1728	2.49	3.25	1.74	6.05	7.79
57	Roasters ..	336	1,391	4.14	Oct. 24 to Nov. 3.	11	604.0	309	1,345	4.35	93	6.7	322.5	0	.1702	6.49	4.28	4.60	15.77	20.37
58	Springers .	800	2,172	2.72	Oct. 25 to 31...	7	939.0	769	2,447	3.18	355	16.3	784.5	0	.1710	2.65	6.62	1.86	6.44	8.30
59	Roasters ..	400	1,365	3.41	Oct. 26 to Nov. 4.	10	613.0	322	1,433	4.45	105	7.7	361.0	0	.1698	5.84	4.35	4.14	14.19	18.33
60	...do......	672	2,612	3.89	Oct. 28 to Nov. 6.	10	1,100.0	614	2,730	4.45	317	12.1	643.0	2	.1711	3.47	7.75	2.44	8.43	10.87
61	Springers .	800	2,125	2.66	Oct. 28 to Nov. 4.	8	1,020.0	718	2,389	3.33	385	18.1	759.0	2	.1680	2.65	7.32	1.90	6.44	8.34
62	Roasters ..	672	2,703	4.02	Oct. 30 to Nov. 8.	10	1,034.0	589	2,722	4.62	285	10.5	630.5	2	.1639	3.63	7.60	2.67	8.82	11.49
63	Springers..	800	1,930	2.41	Oct. 30 to Nov. 5.	7	965.0	785	2,446	3.12	558	28.4	792.5	1	.1739	1.73	6.69	1.20	4.20	5.40
64	Broilers...	400	1,090	2.73	Nov. 2 to 9....	8	474.0	384	1,282	3.34	229	21.0	392.0	0	.1510	2.07	4.90	2.14	5.05	7.19
65	Roasters ..	672	2,598	3.87	Nov. 2 to 10...	9	903.0	663	2,661	4.01	188	7.2	667.5	2	.1502	4.80	9.39	4.99	11.71	16.70
66	Broilers...	400	965	2.41	Nov. 4 to 10...	7	415.0	386	1,151	2.98	258	26.7	393.0	1	.1508	1.61	4.30	1.67	3.93	5.60
67	Roasters ..	672	2,507	3.73	Nov. 5 to 13...	9	892.0	606	2,673	4.41	274	10.9	639.0	7	.1550	3.26	8.99	3.28	7.95	11.23

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[1] The difference between the "Number in" and the "Number out" does not represent the dead birds in this table, as the "cripples" were removed during the feeding period and their dressed weight credited to the lot when it was killed.

[2] For the purpose of computing results, the "Average number fed" is arrived at by adding the "Number in" and the "Number out" and dividing by 2, thus getting an average.

TABLE II.—*Details of feeding experiment B*—Continued.

Lot.	Class.	Number in.	Total weight.	Average weight.	Dates fed.	Days fed.	Total feed.	Number out.	Total weight.	Average weight.	Total gain.	Per cent gain.	Average number fed.	Dead.	Average grain daily per head.	Grain per pound of gain.	Total cost of labor.	Cost of labor per pound of gain.	Cost of feed per pound of gain.	Total cost per pound of gain.
		Head.	*Pounds.*	*Pounds.*			*Pounds.*	*Head.*	*Pounds.*	*Pounds.*	*Lbs.*	*Per ct.*	*Head.*		*Pounds.*	*Pounds.*	*Dollars.*	*Cents.*	*Cents.*	*Cents.*
68	Broilers...	400	941	2.35	Nov. 6 to 13...	8	461.0	371	1,112	3.00	211	22.4	385.5	2	0.1493	2.18	4.82	2.28	5.32	7.60
69	Roasters..	672	2,588	3.85	Nov. 9 to 17...	9	934.0	615	2,735	4.45	371	14.3	643.5	2	.1613	2.52	9.05	2.44	6.15	8.59
70	Broilers...	800	1,845	2.31	Nov. 10 to 16..	7	919.0	761	2,217	2.91	459	24.9	780.5	4	.1682	2.00	8.54	1.88	4.88	6.74
71	Roasters..	672	2,741	4.08	Nov. 11 to 18..	8	858.0	615	2,660	4.33	143	5.2	643.5	0	.1667	6.00	8.05	5.63	14.64	20.27
72	Springers.	400	881	2.20	Nov. 14 to 20..	7	462.0	382	1,197	3.13	357	40.5	391.0	2	.1688	1.29	4.28	1.20	3.15	4.35
73	Roasters..	672	2,832	4.21	Nov. 13 to 22..	10	1,068.0	616	2,862	4.65	217	7.7	644.0	1	.1657	4.92	10.07	4.64	12.00	16.64
74	Springers.	400	1,068	2.67	Nov. 16 to 22..	7	450.0	389	1,275	3.28	236	22.1	394.5	3	.1630	1.91	4.32	1.83	4.66	6.49
75	Roasters..	1,008	4,135	4.10	Nov. 17 to 24..	8	1,277.0	954	4,346	4.56	397	9.6	981.0	3	.1627	3.22	12.27	3.09	7.86	10.95
76	Springers.	400	1,037	2.59	Nov. 18 to 25..	8	490.0	387	1,148	2.97	143	13.8	393.5	1	.1556	3.43	4.92	3.44	8.37	11.81
77	...do......	400	989	2.47	Nov. 20 to 27..	8	486.0	382	1,135	2.97	211	21.3	391.0	2	.1552	2.30	4.89	2.32	5.61	7.93
78	Roasters..	672	2,726	4.06	Nov. 20 to 27..	8	811.0	635	2,881	4.54	298	10.9	653.5	2	.1552	2.72	8.17	2.74	6.64	9.38
79	Springers.	400	1,065	2.66	Nov. 22 to 28..	7	422.0	392	1,200	3.06	154	14.5	396.0	0	.1523	2.74	4.33	2.81	6.69	9.50
80	Roasters..	672	2,793	4.16	Nov. 23 to 29..	7	721.0	647	2,880	4.45	162	5.8	659.5	1	.1560	4.45	7.22	4.46	10.86	15.32
81	...do......	672	2,698	4.01	Nov. 23 to 29..	7	722.0	650	2,755	4.24	143	5.3	661.0	0	.1560	5.05	7.23	5.06	12.32	17.38
82	...do......	672	2,694	4.01	Nov. 27 to Dec. 2.	6	603.0	602	2,705	4.49	182	6.8	637.0	0	.1578	3.31	5.97	3.28	8.08	11.36
83	Springers.	672	2,486	3.70	Nov. 29 to Dec. 5.	7	737.0	651	2,616	4.02	245	9.9	661.5	2	.1591	3.01	7.24	2.96	7.34	10.30

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